Cardiovascular effects of *Leonotis leonurus* extracts in normotensive rats and in isolated perfused rat heart

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ABSTRACT

Cardiovascular effects of *Leonotis leonurus* extracts in normotensive rats and in isolated perfused rat heart.

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This cardiovascular effects of the aqueous leaf extract and a fraction of the methanol extract of *Leonotis leonurus,* a plant commonly used in traditional medicine in South Africa for the treatment of hypertension and other cardiac problems, was tested on anaesthetized normotensive male Wistar rats and isolated perfused rat hearts. Mean change in systolic pressure, diastolic pressure, mean arterial pressure and heart rate in the anaesthetized normotensive male Wistar rats, and mean percentage change in systolic pressure, diastolic pressure, developed pressure, coronary flow, aortic output, cardiac output and heart rate in the isolated perfused rat heart was recorded.

In anaesthetized normotensive male Wistar rats, the crude aqueous extract, administered in the dose range of 0.5mg – 7.0mg intravenously, significantly ($p<0.05$) increased systolic pressure and mean arterial pressure. There was a non-significant ($p>0.05$) decrease in diastolic pressure at the 0.5mg and 1.0mg dose, while the 2.0mg to 7.0mg doses significantly increased diastolic pressure. Heart rate was non-significantly increased at the 0.5mg and 1.0mg doses, while at higher doses (2.0mg – 7.0mg) there was a significant decrease in heart rate by the crude aqueous extract.

Administered after pre-treatment with Atenolol (2.0mg), there was a significant decrease in the increase in systolic pressure by the crude aqueous extract at 0.5mg, 1.0mg, 6.0mg and 7.0mg. At other doses, the reduction in effect of the crude aqueous extract by Atenolol was non-significant. There was also a further decrease in diastolic pressure at the 0.5mg and 1.0mg doses, while from the 2.0mg to 7.0mg dose, the initial increase in diastolic pressure produced by the crude aqueous extract was reversed to a decrease when the animal was pre-treated with Atenolol. The increase in mean arterial pressure was also reversed at low doses (0.5mg and 1.0mg), while at higher doses (2.0mg – 7.0mg), there was a reduction in the increase in mean arterial pressure after pre-treating the animals with Atenolol (2.0mg). The increase in heart rate at the 0.5mg and 1.0mg doses was significantly reversed to a decrease, while a further decrease in heart rate at other doses was observed. This was significant between 2.0mg and 5.0mg doses.

Pre-treatment with prazosin (60μg/kg) significantly reduced the increase in systolic pressure produced by the crude aqueous extract on anaesthetized normotensive male Wistar rats. The reduction in diastolic pressure at 0.5mg dose was significantly increased, while the increase at 1.0mg and 2.0mg doses there was a significantly reversal of the increase in diastolic pressure to a decrease. At higher doses, the increase in diastolic pressure was reduced when prazosin was pre-administered. The increase in mean arterial pressure at 0.5mg and 1.0mg was significantly reversed to a decrease by prazosin, while the increase obtained at higher doses of the crude aqueous extract was significantly reduced. The increase in heart rate at 0.1mg and 1.0mg doses was reduced, while the increase in heart rate at 2.0mg was reversed to a decrease. At higher doses of the crude aqueous extract, there was a significantly increase in the decrease in heart rate produced by the crude aqueous extract with the pre-administration of prazosin in anaesthetized normotensive male Wistar rats.

In isolated perfused rat hearts, a 0.01mg/ml dose of fraction C significantly increased systolic pressure and developed pressure, while significantly reducing diastolic pressure. Coronary flow, aortic output, cardiac output as well as heart rate were also significantly increased by this dose. Co-administration of the same dose of fraction C with 0.07mg/ml Atenolol produced a significant reduction in the increase in systolic pressure by fraction C. There was also a significant reduction in the decrease in diastolic pressure, but the reduction in the decrease in developed pressure was not significant. Increase in coronary flow and aortic output were significantly reversed to a decrease, while the increase in cardiac output was significantly reduced. Heart rate was significantly reversed from an increase to a decrease.

The data obtained indicate that Leonotis leonurus contains cardioactive compounds, with specific cardiovascular activity depending on the dose and the preparation administered. The crude aqueous extract had a positive chronotropic ad inotropic effect at low doses (0.5mg and 1.0mg), and a negative chronotropic and positive inotropic effect at higher doses (2.0mg – 7.0mg) in the anaesthetized male wistar rat. Fraction C from the methanol extract had a positive chronotropic and positive inotropic effect at all doses (0.01mg – 0.05mg) in the anaesthetized male wistar rat and in isolated perfused rat heart.

DECLARATION

I declare that "Cardiovascular effects of *Leonotis leonurus* extracts in normotensive rats and in isolated perfused rat heart" is my own work, that it has not been submitted for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Full name: **Obikeze, kenechukwu chibuzo**

Signed: _____

Date : 23/02/05

DEDICATION

Thanks be to God for making this dream a reality and for giving me the strength to see this through. Thanks also to my parents, Prof. and Dr. Obikeze for their steady support through the years, and to Binche, Zume, Dennanna and Nony you guys are great.

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CHAPTER ONE

INTRODUCTION AND LITRATURE REVIEW

1.1 INTRODUCTION

Man has practiced the use of plants, animals and naturally occurring minerals as medicines for the cure of diseases for centuries. It is an evolving practice recorded in both folklore and books of early practitioners. Typical recipes for cure of diseases constituted of decoctions, poultices, ointments and solutions of plants, animal parts and minerals (Costa-Neto, E. .M. 1999; Noumi *et al* 1999). Though a lot of these remedies have disappeared with time, some still exist in present times and are used to this day for the treatment of diseases by traditional medicine practitioners of all cultures.

Plants, animals and minerals were once the only source of medicines in the world. Early medications started out as decoctions of plant (and sometimes animal or mineral) parts, however with the discovery of pure drugs like quinine, atropine, and reserpine from plants, the trend leaned towards the production of pure drugs from plant and animal precursors. Though this trend changed with the advent of purely synthetic drugs, the pharmacological potential of plants still abounds as only about 90 species out of the 250 000 species of higher plants on the planet have or are presently in use for drug production (Chadwick, D.J., Marsh, J. (Eds). 1990). Even in the realm of traditional medicine practice, only a fraction of the 250 000 species of higher plants have found use as therapeutic agents (Chadwick, D.J., Marsh, J. (Eds). 1990).

At present, despite the abundance of synthetic drugs, a significant proportion of the population of developing countries depend on traditional medicines for their health care needs. According to world health organization (WHO) estimates, 60% of the world's population depend on traditional medicine, and 80% of the population in developing countries depend almost completely on traditional medical practices for their primary health care needs (Zhang X. 2000; Chadwick, D.J., Marsh, J. (Eds). 1990). Of the majority of traditional medicine users residing in developing countries, about 15-20 million reside in Southern Africa alone (Duncan *et al* 1999).

South Africa is a country with a large biodiversity of over 30 000 higher plant species. Of this lot, approximately 3000 species of higher plants are presently used as medicines by

an estimated 60% of the population (Mander *et al* 1997). In addition to this, it is estimated that 80% of the population consult traditional healers first for their health problems (Duke, J.A. 2001). It is no surprise therefore that as of 1996, an estimated 20 000 tonnes of over 700 medicinal plant species were traded in South Africa every year, with a market value of some $60 million (approximately R450 million)(Mander *et al* 1997). Unfortunately however, of this huge amount of traditional plants in use as traditional medicines, most of the times in conjunction with orthodox medicines, only about 350 commonly used and traded species have undergone chemical investigations (Mander *et al* 1997).

In the developing world, there is an increase in mortality and morbidity from cardiovascular diseases, possibly due to changes in lifestyles of people (Tabuti *et al* 2003). The search for the cure for hypertension and other cardiovascular diseases continues, with the promise new, more effective drugs on the way, but for most sufferers in developing countries, hope lies in the direction of herbal drugs because of their promise of a cheap and readily available cure. Due to its high profile as being the number one cause of deaths, a lot of the research into traditional medicine has focused on their use in the treatment of cardiovascular diseases, especially hypertension. Studies of the remedies in traditional medicine by various researchers have shown an extensive use of plants in the treatment of cardiovascular diseases. For example, the Chinese used *Ginko biloba* for the treatment of heart diseases, *Stephania tetandra* for the treatment of hypertension, the root of *Lingusticum walliichii* as a circulatory stimulant, a hypotensive drug and sedative, and *Uncaria rhynchophylla* for hypertension (Mashour *et al* 1998; Mahady, G. 2002). Noumi and co-workers came up with about 20 different plants used in Bafia, Cameroon for the treatment of hypertension, Blanco and co-workers in their study found traditional plants in use traditionally as diuretics, cardiotonics, antihemorrhagics, antioxidants, and vasodialatory agents, while Van Wyk in his book mentions the use of *Leonotis leonurus* amongst other plants for the treatment of hypertension. (Noumi *et al* 1999; Blanco *et al* 1999; Van Wyk et al. 2000).

Taking into consideration the present shift by the WHO and the AU towards the integration of traditional medicines into the national health plan, as evident in the recent traditional medicines bill in South Africa, there is an increasing need for chemical as well as pharmacological research into traditional medicines. Though the field of research into

traditional medicines has been on the increase, there are yet a large number of plants in use as traditional medicine whose pharmacologic and toxic effects and well as possible harmful interactions with foods and other drugs are unknown. Although traditional medicine is the primary form of therapy in many parts of the world, it suffers from many shortcomings. As indicated previously, there have been no studies on the efficacy of most traditional remedies, most of the claims made of the therapeutic effectiveness of these medicinal plants are made by the traditional healers themselves, and the understanding of the pathophysiology of the disease states they claim their remedies treat is very limited if non-existent (Kale, 1995; Sofowora, 1982). Traditional medicines, though they are effective and potent medicines for quite a lot of diseases, require evaluation by scientific methods in order to be used to their full effect. Despite the growing popularity of traditional medicines in the modern world, little attention has been paid to their possible side effects, toxicities and interactions. Much of this may be due to the fact that most herbal medicines are ubiquitous. Furthermore, there is little or no information on animal experiments or clinical trials for most herbal drugs presently in circulation (Tomassoni, A. J. and Simone, K. 2001). Another possible reason for the paucity of information on drug-herb interactions is the lack of information on the mechanisms whereby most herbal drugs act. Since no regulatory body is in place to oversee the standards of traditional medicines, a lot of life-threatening and even lethal interactions and adverse reactions by these drugs go unnoticed. Until the recent upsurge in interest in traditional medicine and its recognition by the WHO as a compliment to orthodox medicine, most practitioners operated at the fringes of the healthcare system, and were consulted by patients who were most of the time also on orthodox medication. Traditional practitioners had little knowledge of orthodox medicine and diseases, while medical practitioners took no account of concomitant use of traditional medicine with prescriptions. There is currently little or no scientific evidence as to the mode of action of most of the traditional remedies currently in use. Adverse effects are seldom reported and little effort has been made to research into this area of growing concern. Traditional medicines, as effective and potent medicines, require evaluation by scientific methods in order to be used to their full effect.

This study examined the effects of the crude aqueous extract of *Leonotis leonurus* on the anaesthetized male wistar rat. The effect of fraction C obtained from column chromatography of the methanol extract of the leaves, using ethyl acetate: Hexane (1:4) as the mobile phase, on the anaesthetized male wistar rat and on the isolated perfused rat

heart was also examined. This study also examined the effect of negative chronotropic drug – atenolol, and vasodilator – prazosin on the cardiovascular effect of the aqueous extract of the leaves on anaesthetized male wistar rats. Finally, the effect of a 0.01mg/ml solution of fraction C containing atenolol (0.07mg/ml) on the isolated perfused rat heart was also examined.

Chapter one of this thesis contains an introduction to the topic matter. It contains a brief overview of the evidence of the use of traditional medicines both past and present in various disease states. Plant remedies in use for various diseases would be examined, with an emphasis on those in use for cardiovascular diseases. A description of the plant *Leonotis leonurus*, its use in traditional medicine as well as the results of various researches into its composition and pharmacologic effects will be described. Chapter two looks at the cardiovascular system. It describes the functional physiology of the heart, mechanisms of intrinsic and extrinsic regulation of the heart and factors affecting normal cardiac function. Chapter two also contains an overview of drugs affecting cardiac function and a description of some of the receptors involved in the mediation of the effects of these drugs on the cardiovascular system. The two cardiovascular research models used are also described and the parameters measured explained. Chapter three lists the material used and describes the methods used in the study. The material used in the extraction of the plant and the cardiovascular models are listed or described. The methods used in the aqueous and methanol extraction of the plant leaves, as well as chromatography of the methanol extract is described. The experimental protocol for both in vivo and in vitro experiments is also described. Chapter four contains illustrations and a discussion of the results obtained. It also contains the conclusions derived from the study and recommendations. Chapter five states the conclusions drawn from the results and also recommendations on further research work.

1.2 LITRATURE REVIEW

At the time when modern methods of synthesizing drugs were not available, plants and other naturally occurring substances were the only source of drugs for mankind. Evidence of extensive use of plants as drugs can be found in traditional Chinese, Indian ‘ayurveddic’, and Pakistani ‘unani’ literature (Chadwick, D.J. and Marsh, J. (Eds). 1990; Mahady, G.B. 2002; Pemberton, R.W. 1999). Since most African societies had no written

language, important traditional medical recipes were preserved via oral traditions and are found in the folklore of these societies (Williamson et al. 1996). Plants have shown usefulness not only as sources of drugs, but also have been employed as markers in the elucidation of some physiological processes in the body like the determination of muscarinic and nicotinic receptors in the body (Williamson et al. 1996). Though in present times most of the drugs manufactured are of synthetic origin, approximately 25% of drugs which have been used worldwide are or were derived from plants and used either in their pure form, (e.g. morphine, codeine, vincristine and vinblastine) or as they appear in nature (e.g. teas, decoctions and extracts of leaves, barks, fruits, flowers, e.t.c.) (Kutchan M. T. 1995). Plants have also served as models from which wholly synthetic drugs are designed. Examples include; tropicamide derived from atropine an alkaloid obtained from *Atropa belladonna*, chloroquine derived from quinine, and procaine and tetracaine derived from cocaine. Interestingly plants also find use in cases were conventional therapy is not effective; examples include the use of *Silybum marianum* to prevent liver damage from poisoning by death cap mushroom and infectious hepatitis, the use of immune stimulants from coneflower for viral infections, and the controversial use of *Cannabis sativa* to treat pain and nausea in cancer and cancer chemotherapy (Kutchan M. T., 1995). The increase in reliance on traditional medicines, the rejuvenation of interest in plant medicines as a potent source of new drugs for chronic diseases and the need for a pharmacologic profile of plants in use as traditional medicines have lead to a recent increase in research into the pharmacologic effects of plants that are used in traditional medicine.

1.2.1. CARDIOVASCULAR EFFECTS OF TRADITIONAL PLANTS

Like therapy for all other ailments, therapies for cardiovascular diseases abound in most traditional medicine systems. While some of these were targeted ambiguously at ‘heart problems’, some of the therapy was more specific for congestive heart failure (CHF), systolic hypertension, angina pectoris, arteriosclerosis, cerebral insufficiency, venous insufficiency and arrhythmia (Van Wyk *et al* 2000; Mashour *et al* 1998). Recently, a lot of work has been done in the area of the pharmacological evaluation of these plants for pharmacological/ physiological activity. Though this has been going on for quite a while, the majority of plants used in herbal medicine are yet to be evaluated pharmacologically.

Various methods and models have been used by various investigators to determine the cardiovascular activity of different plants used in traditional medicine.

In a study done by Mashour and co-workers, tetrandrine, an alkaloid extract of *S. tetrandra*, was shown to sustain hypotensive effects for more than 48 hours after a 25 or 50mg oral dose to a rat. Its effects were quite similar to those of verapamil, a calcium ion channel antagonist also used in the study (Mashour *et al* 1998). The harmala alkaloids harmine, harmaline and harmalol were also investigated for cardiovascular action in isolated perfused rat hearts, intact dogs and intact normotensive anaesthetized dogs. All three alkaloids were found to decrease heart rate, increase developed pressure, peak aortic flow and myocardial contractile force in intact normotensive anaesthetized dogs. Harmine reduced systemic arterial blood pressure and total peripheral vascular resistance, harmaline caused decreases frequently followed by a secondary increase in systemic arterial blood pressure and total peripheral vascular resistance, and harmalol had inconsistent effects on these two parameters. A direct negative chronotropic effect was produced by the harmala alkaloids in the isolated perfused rat heart and in the intact dog (Aarons *et al* 1977).

The cardioactive effects of an aqueous extract obtained from the leaves of the traditional Aboriginal medicinal plant of *Eremophilia alternofilia* was investigated the on isolated hearts of normotensive rats using the Langendorff heart perfusion model (Pennacchio *et al* 1995). From the results obtained, the crude aqueous extract of *E. alternofolia* leaves mediated an initial, but transient, positive inotropic effect followed by an immediate negative inotropic effect accompanied by an increase in heart rate and coronary perfusion rate. These effects were not blocked with the administration of phentolamine was perfused. Dried powdered bulbs of *Allium sativum* produced a dose-dependant diuretic and natriuretic response in anaesthetised dogs when administered intragastrically in a study done by Pantoja and co-workers (Pantoja *et al* 1991). Similar results were also obtained when chromatographic fractions of the plant were administered intravenously to anaesthetized rabbits (Pantoja *et al* 1996). Though the fractions showed diuretic-natriuretic effects, and a gradual decrease in heart rate at high doses, electrocardiogram (ECG) and arterial blood pressure remained at normal levels. The effects of garlic dialysate on diastolic blood pressure, heart rate, and electrocardiogram (ECG) of anaesthetized dogs as well as its effects on the frequency of contraction and tension of the

isolated rat atria were investigated by Martin and co-workers (Martin *et al* 1992). Intravenous injection of garlic dialysate (16.8mg/kg - 67.2mg/kg) produced a rapid and dose-dependent hypotensive and negative chronotropic effects in anaesthetized dogs. The cardiovascular effects of Visnagin, an active principle isolated from the fruits of *Ammi visnaga,* used for the treatment of angina pectoris, have also been investigated. Duarte and co-workers investigated its effects on systolic blood pressure and heart rate in anaesthetized male wistar rats and found that Visnagin lowered the blood pressure of the anaesthetized normotensive rat, but had no effect on the heart rate (Duarte *et al* 1999).

In South Africa, Duncan and co-workers investigated the aqueous and ethanol extracts of twenty plants used by traditional healers in South Africa in the treatment of hypertension for anti-hypertensive properties, using a modification of the angiotensin converting enzyme (ACE) assay method developed by Elbl and Wagner (Duncan *et al* 1999). A hit rate of 65% was achieved with the highest inhibition of 97% achieved by extracts of the leaves of *Adenopodia spicata*. Extracts of *Agapanthus africanus, Agave Americana. Clausena anisata, Dietes iridioides, Mesembruanthemum spp., Stangeria eriopus* and *Tulbaghia violacea*, exhibited inhibition greater than 70% and five other plants exhibited inhibition over 50%. There was also little difference in the overall hit rate between aqueous and ethanol extracts, although in most cases there were marked differences in activity between aqueous and ethanol extracts from the same species (Duncan *et al* 1999). In a study done by Mugabo and Njagi, an infusion of the crude aqueous leaf extract of *Leonotis leonurus* had no significant effect on systolic and diastolic in anaesthetized male Wistar rats. In addition to this, previous studies by Mugabo on the crude aqueous extracts of the plant indicated that the plant had a positive chronotropic and a negative inotropic effect on the isolated perfused rat heart, and recommended further studies on isolating the active components of the plant (Njagi *et al* 2001; Mugabo *et al* 2002).

1.2.2. *LEONOTIS LEONURUS*

Leonotis leonurus is a shrub indigenous to Southern Africa and found over large parts of South Africa. It is common at forest margins, on rocky hillsides and riverbanks and in tall grasslands of the Eastern and Western Cape Provinces, Kwazulu-Natal and Mpumalanga (Van Wyk et al., 2000). The plant is commonly known as wilde dagga in Afrikaans, wild dagga in English, umunyane in Zulu, lebake in Sotho and umfincafincane in Xhosa (Watt

et al., 1962 and Van Wyk et al., 2000). It has thick wooden base, pale brown branches and grows between two and five meters in height. The leaves are hairy, long and narrow with serrated upper edges, and are arranged opposite each other on the stems. The flowers are bright orange in colour and tubular in shape, and are arranged in circles along branch ends (Van Wyk et al. 2000).

Figure 1.1: *Leonotis leonurus* (L.)

Leonotis leonurus is a shrub well known in traditional medicines. It has been documented for use in treating all kinds of diseases from influenza, tuberculosis, jaundice, muscular cramps, skin diseases, sores, bee and scorpion stings, to menstrual disorders and hypertension (Van Wyk et al. 2000).

Various compounds have been isolated from the *Leonotis* species; these include leonurine a mildly psychoactive alkaloid isolated from *Leonotis. sibricus* as well as *Leonotis. leonurus,* a volatile oil, and several unusual diterpenoids (labdane type lactones) (Van

Wyk et al. 2000). *Leonotis leonurus* is also known to contain marrubin (a labdane type lactone also found in *Marrubium vulgare*), C-13 epimeric premarrubiin (a diterpene spiro ether), and two labdane terpenoids known as compounds X and Y. (Rivett, D.E.A. 1984; Hutchings et al. 1996; Duke, J.A. 2001).

Chemical compounds isolated from the leaves of the plant include; a) Two (2) phenolic compounds - ($C_9H_{1O}O_3$ & $C_8H_{10}O_5$), (b)A resin, c)A reddish oil (Marrubin) and, (d)Two (2) diterpenes ($C_{20}H_{28}O_5$ & $C_{20}H_{28}O_3$) (Muhizi, T. 2002; Duke, J.A. 2001; Hutchings et al., 1996). Histochemical Characterization of the oleoresin produced by peltate trichomes of leaves of *Leonotis leonurus* revealed terpenoids and Flavonoid aglycones (Ascensao, L. and Marques, N. 1997). Physicochemical analysis of acetone and hexane extracts of the powdered leaves of the plant by Muhizi yielded six yet to be identified compounds denoted A to G, while the aqueous extract of the leaves tested positive for alkaloids, saponins and tannins (Muhizi, T. 2002).

1.2.3. TRADITIONAL USE OF *LEONOTIS LEONURUS*

Leonotis leonurus was and is still extensively used in traditional medicine all over the world. An undefined preparation of the stem and leaves of the plant is reported to be used for the treatment of menstrual disorders in the Dominican Republic (Ososki et al. 2002). The plant is amongst twenty-six others used to treat hypertension traditionally in the Bafia region, Cameroon, and Duke reports that various parts of the plants, either as decoctions or inhalations, are used in the treatment of asthma, common cold, epilepsy, leprosy, high blood pressure and other 'cardiac conditions' (Noumi et al, 1999; Duke, J.A. 2001). Infusions of the flowers, leaves or stems are widely used in various other parts of Africa and Mauritius as purgatives and tonics and for influenza, tuberculosis, jaundice, muscular cramps, skin diseases, sores, bee and scorpion stings. Decoctions have also been used for the relief of cardiac asthma, and ointments containing powdered leaves are applied for pain above the eye (Hutchings et al., 1996).

In South Africa, the plant has been in use the early times. Hottentots were particularly fond of smoking it instead of tobacco and used a decoction of the leaf as a strong purgative and as an enemagogue . Early colonists employed a decoction in the treatment of chronic cutaneous eruptions and possibly even in leprosy (Watt et al. 1962). An

infusion of the stem and leaves of the plant is used by the Zulus for coughs and colds in both human beings and stock (Watt et al., 1962). The Zulus also use a cold infusion of the leaf as a nasal douche to relieve headache in febrile attacks. A decoction of the powdered stem or seed was drunk for the relief of hemorrhoids and used as a lotion for sores on the legs and head (Watt et al., 1962). The leaf was smoked as a cure for partial paralysis and a relief from epilepsy (Hutchings et al., 1996; Van Wyk et al., 2000). The leaves or roots are widely used as a remedy for snakebites and also to treat other bites and stings (Hutchings et al., 1996 and Van Wyk et al., 2000). Leaf infusions have been used for asthma and viral hepatitis and teas used as diuretics and for obesity. (Van Wyk et al., 2000; Hutchings et al., 1996; Watt et al., 1962).

1.2.4. CARDIOVASCULAR EFFECT OF *LEONOTIS LEONURUS*

Ethanol extracts of *Leonotis leonurus* was found to be one of the highest inhibitors of cycloxygenase enzyme, when tested along with other plants used in traditional Zulu medicine for the treatment of headaches or inflammatory diseases (Jager, *et al* 1996).

An aqueous extract of *Leonotis leonurus* was tested for anticonvulsant properties against seizures produced in mice by pentylenetetrazole, picrotoxin, bicuculline and N-methyl-DL-aspartic acid, and results showed that the aqueous extract of *L. leonurus* protected some of the animals against seizures induced by pentylenetetrazole, picrotoxin and N-methyl-DL-aspartic acid and also delayed the latency of the seizures. (Bienvenu E. 2001).

The cardiovascular activity of Leonotis leonurus has been previously investigated, and various researchers have come up with different results of the plants effects. A study by Njagi and Mugabo showed that the infusion of a 1mg/ml aqueous extract of the leaves had no significant effect on the systolic and diastolic pressures as well as the heart rate of anaesthetized male wistar rats (Njagi et al 2001). A study of the cardiovascular properties of an aqueous decoction of the leaves of *Leonotis leonurus* by Mugabo et al found it to exhibit positive chronotropic and inotropic effects on isolated male wistar rat hearts (Mugabo et al 2002). In another study by Ojewole however, a dose range of 25 – 800mg/kg of the aqueous leaf extract injected I.V produced significant dose-related decreases in the arterial blood pressures and heart rates of anaesthetized, normal and spontaneously hypertensive rats. He also found out that the hypotensive effect of the leaf extract was more pronounced in the hypertensive rats than in the normal ones (Ojewole, J.A. O. 2003). These results were different from that of Njagi, but could be explained by

the fact that the dose range used by Ojewole was far higher than that used by Njagi. Working with strips of the descending aorta and portal vein, Ojewole also found that the plant extract (LL, 25 - 800 _g/ml) relaxed, vascular smooth muscle contractions induced by bath-applied noradrenaline (NA, 0.1-10 _M)-, and potassium (K_, 5/40 mM)in a non-specific manner. These studies however were not able to determine the mechanism by which the plant exerted its effects, nor was there any attempt to isolate active compounds responsible for the effects observed. Since these studies were carried out either on isolated hearts or isolated tissue, or anaesthetized rats, the question remains as to the effects of the plant on isolated organ preparations and the intact animal. The variations in results so far obtained by researchers bring the questions of whether the observed effects of the plant are dose dependent or are brought about by different compounds.

The need for evaluation of traditional medicines for their pharmacological activity is underlined by study done by Mashour and co-workers on the efficacy and safety of some herbal medicines with effects on the cardiovascular system. The herbs were categorised under the primary diseases they treat, and some of the plants examined included *Datura purpurea* (foxglove), *Adonis microcarpa* and *Adonis vernalis* (adonis), *Asclepias friticosa* (balloon cotton), *Calotropis precera* (king's crown) *Carissa spectabilis* (wintersweet), *Strophanthus hispidus* and *Strophanthus kombe* (strophanthus) and *Thevetia peruviana* (yellow oleander) (Mashour *et al* 1998). All the plant parts of *Thevetia peruviana* including the leaves, seeds, roots, flowers and berries was found to be extremely toxic, with death in humans possible after the ingestion of as little as one oleander leaf.

From the literature it is evident that there is a growing interest in natural and traditional medicines as a source of treatment for various cardiovascular diseases. The literature shows that there is continuing research being done on medicinal or traditional plants used in the treatment of cardiovascular disease. However the objectives of most of such studies has been the evaluation and validation of the claims of the therapeutic uses of plants in traditional medicine, and not the development of these plant remedies into orthodox medicines. Though this may be considered a disadvantage, it must also be noted that the validation or otherwise of these therapy would go a long way in the integration of traditional medicine into the primary healthcare system, and at the same time forestall an avalanche of morbidity and mortality due to the adverse effects as well as the

pharmacologic effects of these herbs. Recent research into possible interactions of herbal drugs with orthodox medicine has shown them not to be as harmless as purported. A wide range of interactions between drugs and plants ranging from the 'common' garlic and ginko to preparations of African, Chinese and Indian medicines has been noted (Fugh-Berman, A. 2000: Villegas et al. 2001). Though a lot of research has been carried out in recent years on authenticating the claims of these medicines, a vast majority of herbal medicines in use remain yet to be evaluated for their treatment claims. More pertinent is the evaluation of the side effects, toxicity and interactions of these herbal medicines. As more and more people embrace the use of traditional medicines especially in the developed world, there is an increased likelihood of interactions between these drugs and orthodox therapy (Awang, D. V. C. and Fugh-Berman, A. 2002: Mahady, G. 2002: Scott, G. N. and Gary, W. E. 2002).

This study focused on the cardiovascular effects of *Leonotis leonurus* because of its potentials as noted in literature for use as therapy in cardiovascular diseases. Though its use in traditional medicine is multifarious as has been observed with most plants, previous research into the cardiovascular actions of the plant proved inconclusive and recommended further research into the plants actions.

1.3 AIMS AND OBJECTIVES

The aims of this project were to determine the cardiovascular activity of extracts of *Leonotis leonurus* and to attempt an elucidation of the mechanism of this action.

The objectives included:

To determine the cardiovascular activity of the aqueous extracts of the leaves of *Leonotis leonurus* using the anaesthetized normotensive rat model.

To test the effect of a chronotropic, an inotropic and a vasodilatory drug on the cardiovascular activity of the aqueous extract of the leaves of *Leonotis leonurus* using the anaesthetized normotensive rat model.

To separate different fractions from an organic extract of the leaves of *Leonotis leonurus* using column chromatography, and to test the fractions for cardiovascular activity using the anaesthetized normotensive rat model and the double- sided working heart model.

To test the effect of a chronotropic and an inotropic agent on the cardiovascular activity of active fractions of the organic extract of leaves of *Leonotis leonurus*.

1.4 HYPOTHESIS

It was hypothesized that the active compound isolated from the leaves of *Leonotis leonurus* had a positive inotropic and positive chronotropic effect on the anaesthetised male Wistar rat, and that this cardiovascular effect was by the action of the active compound on the heart and blood vessels.

CHAPTER TWO

THE CARDIOVASCULAR SYSTEM

2.1. ANATOMY AND PHYSIOLOGY

The cardiovascular system is made up of the heart, blood vessels and blood. Its extensive layout of arteries, veins, arterioles, venules and capillaries connect to the various tissues of the body. The main function of this system is to deliver nutrients to the organs, tissues and cells of the body and to remove waste products of metabolism from their vicinity.
Blood from the head and the body collects through a network of venules and veins into the superior and inferior vena cava, and from there into the right side of the heart. From the right ventricle the blood is pumped via the pulmonary circulation through the lungs and back into the left side of the heart, from where it is pumped back into the systemic circulation. At the center of the cardiovascular system is the heart - a four chambered synchronized double pump that circulates blood between the pulmonary and systemic circulations. The blood vessels – arteries, veins, arterioles, venules and capillaries serve as a channel for the circulating blood. This chapter discusses the anatomy and physiology of the heart, cardiac function and factors affecting cardiac function, as well as a brief overview of some of receptors in the cardiovascular system. The animal models used in the study are also described.

2.1.2 THE HEART

2.1.1.1 Description

The mammalian heart is a muscular organ divided into four distinct chambers – two atria and two ventricles. It is enclosed in a double-layered sac made up of a tough, fibrous outer layer (fibrous pericardium) and a thin transparent inner layer (serous pericardium). The part of the serous pericardium lining the fibrous pericardium is called the parietal pericardium and the part covering the heart surface is known as the visceral pericardium. Between these two is the pericardial cavity, filled with pericardial fluid, which helps reduce friction as the heart moves within the pericardial sac. The heart wall itself consists of three layers of tissue – the epicardium, the myocardium and the endocardium. The epicardium lines the outer surface of the heart, while the endocardium lines the inner

surfaces of the heart chambers. In between these is the thick middle layer – the myocardium. This layer is composed of cardiac muscle cells and is responsible for the contraction of the heart.

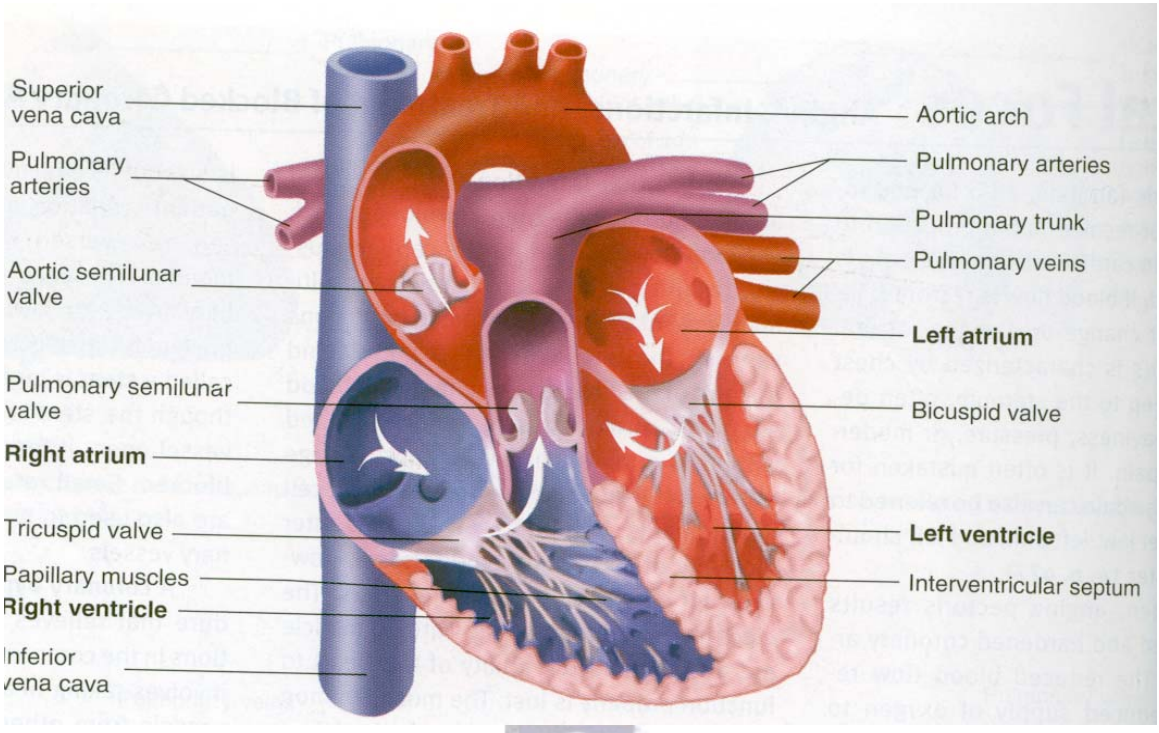

Figure 2.1: Cross-section of the mammalian heart (Seeley, R.R. *et al* 2003).

The atria are thin –walled low-pressure chambers and serve more as reservoir conduits for blood into the ventricles. The ventricles are much more thickly walled, developing high pressures required to pump blood out of the heart and around the body. The right ventricle, which is thinner-walled than the left ventricle, develops one-seventh the mean pressure of the left ventricle. Separating the atria from the ventricles is a large coronary sulcus which runs obliquely round the heart. The anterior intraventricular sulcus and the posterior intraventricular sulcus extend from the coronary sulcus and divide the ventricles into left and right. Separating the atria from the ventricles are valves of flexible endothelium-covered fibrous tissue, which serve to maintain a unidirectional flow of blood through the heart. There are two types of valves in the heart – the atrioventricular valves and the semilunar valves. Atrioventricular valves control the flow of blood between the atria and the ventricles. The tricuspid valve controls blood flow between the right atrium and right ventricle while the mitral valve controls the flow of blood between the left atrium and the left ventricle are. The valves controlling the flow of blood out of

the right ventricle, into the pulmonary artery, and also out of the left ventricle, into the aorta are known as semilunar valves.

Located behind two of the three cusps of the semilunar valves are the orifices of the left and right coronary arteries. These vessels supply the myocardium with blood. The smaller of the two is the right coronary artery probably because it does not supply a large area of the myocardium with blood. It extends to the posterior part of the heart. The intraventricular artery, a major branch of the left artery, supplies blood to most of the anterior parts of the heart, while the left marginal artery (a branch of the left coronary artery) supplies the lateral wall of the left ventricle. The right marginal artery is a larger branch of the right coronary artery and supplies the lateral wall of the right ventricle. The posterior interventricular artery supplies the posterior and inferior parts of the heart with blood. The great cardiac vein drains the left side of the myocardium, and a small cardiac vein drains the right side. These veins meet toward the posterior part of the coronary sinus, which in turn empties in the right atrium. Coronary flow accounts for 5% of the total cardiac output at rest and 8% of total output during exercise.

The heart is a special organ in the body in that it possesses autorhythmicity. The heart contains muscle cells specially modified to spontaneously generate and rapidly transmit electrical impulses. These special cells are grouped into two nodes and a conducting bundle. The two nodes are located within the walls of the right atrium; with the sinoatrial (SA) node medial to the opening of the superior vena cava and the atrioventricular (AV) node medial to the right atrioventricular valve. The SA node is known as the pacemaker of the heart because of its ability to generate spontaneous action potentials at a greater frequency than the rest of the heart. Action potentials generated from the SA node are conducted rapidly to the AV node via preferential conduction pathways. Arising from the AV node is a conducting bundle (atrioventricular bundle), which passes through the fibrous skeleton to reach the intraventricular septum where it divides into two (left and right) branches that extend to the apices of the left and right ventricles respectively. Inferior branches of the bundle branches are modified to conduct action potentials more rapidly than all other cardiac muscles so as to deliver the action potential simultaneously to all parts of the ventricles. These fibers are known as Purkinje fibers.

2.1.1.2 Cardiac function

In carrying out its function as a double rhythmic pump, the heart goes through a repeated cycle of rhythmic contraction and relaxation of the atria and ventricles from internally generated action potential. When the heart beats in resting conditions, it takes approximately 0.04 second for the action potential to travel from the pacemaker (SA node) to the AV node. The action potentials are propagated slowly through the AV node and take about 0.11 second to get to the AV bundle. This total delay of 0.15 second is to allow for complete atrial contraction before ventricular contraction begins. After passing from the AV node to the conduction bundles, conduction velocity speeds up and the action potential passes through the left and right bundle branches, through the different Purkinje fibers to penetrate the ventricular myocardium and cause ventricular contraction (Seeley, R.R. *et al* 2003).

The cardiac cycle begins at the time of ventricular contraction, known as ventricular systole. At the beginning of the ventricular systole, ventricular contraction causes the closure of the atrioventricular (AV) valves. The semilunar valves, which were closed during the preceding diastole, also remain closed. Continued ventricular contraction increases the intraventricular pressure until it exceeds the pressure in the pulmonary trunk and aorta (80mmHg in human and 76-97mmHg in a 300gm rat), the semilunar valves are forced open and blood is pumped out of the heart into the pulmonary and systemic circulations (Seeley, R.R. *et al* 2003). At the beginning of diastole, the ventricles relax and blood flowing back to the ventricles forces the closure of the semilunar valves. The AV valves, which had remained closed until this stage, then open causing the passive filling of the relaxed ventricles from the atria. This is followed by the contraction of the atria, completely emptying their contents into the relaxed ventricle. This is the last stage of the cycle, after which a new cycle begins with the ventricular systole. Blood supply to ventricular muscle mass is essential for efficient cardiac function, and considering the fact that contraction of the ventricle itself impedes coronary blood flow, most of the blood is delivered during diastole.

2.1.1.3 Regulation of cardiac function

The heart plays a major role in the maintenance of homeostasis in the body by varying the rate and force at which it pumps. The rate, force and total amount of blood pumped

by the heart is controlled by either intrinsic or extrinsic regulatory mechanisms. In addition to these mechanisms, it has been shown experimentally that other factors such as changes in temperature, pH, oxygen and carbon dioxide content of the blood, as well as age health status would also affect cardiac function. The effect of these factors is more evident in isolated heart preparations where small changes in parameters such as temperature, pH, and oxygen content of the perfusion fluid would elicit large changes in cardiac function.

Intrinsic regulatory mechanism

Intrinsic regulation of the heart refers to regulation that comes as a result of the normal function of the heart, and is independent of neural or hormonal regulation. The heart adapts to changing hemodynamic environment by mechanisms that are intrinsic to the myocardial muscles. By far the most important aspect of intrinsic adaptation of cardiac function is the role the resting length of the myocardial fibers of the left ventricle play in determining cardiac output. This form of adaptation is described by starling's law of the heart and is known as the Frank-Starling mechanism. During diastole in the cardiac cycle, blood flows into the right atrium from the inferior and superior jugular veins. This is known as venous return. As venous return increases, end-diastolic volume (the amount of blood in the ventricles at the end of diastole) increases and the stretch of ventricular muscle walls (also known as the preload) increases. Frank in 1895 and Starling in 1914 noted that an increase in preload caused the cardiac muscle fibers to contract with greater force, thus producing a greater stroke volume and an increased cardiac output, while a reduced preload caused a decrease in the force of contraction of the cardiac muscle fibers, decreased the stroke volume and thus decreased cardiac output (Seeley, R.R *et al* 2003). The afterload – the aortic pressure the contracting ventricles have to overcome to move blood into the aorta - plays little role in intrinsic regulation. Intrinsic regulation is present both in *in vivo* and *in vitro* preparations, but it plays a greater role in cardiac regulation in isolated heart preparations where the effects of catecholamines are more or else nonexistent. On the other hand, the effects of extrinsic regulation are felt only in *in vivo* preparations.

Extrinsic regulatory mechanisms

Extrinsic regulation involves both neural and hormonal control of heart function. This regulation of the heart functions to keep the blood pressure, blood oxygen and carbon

dioxide levels, and blood pH within normal physiological ranges. Neural control is by sympathetic and parasympathetic enervation of the heart while adrenaline and nor-adrenaline excreted by the adrenal medulla account for the hormonal control of the heart. The Atria and junctional tissue in the heart are richly innervated by parasympathetic and sympathetic fibers, while the ventricles are predominantly innervated by sympathetic fibers. Sympathetic and parasympathetic nerve innervations influence both the heart rate and the stroke volume, though sympathetic stimulation has the greater effect on the heart. Sympathetic stimulation can increase cardiac output by 50% - 100% of the resting values, while parasympathetic stimulation can cause only a 10% - 20% decrease. Neural and hormonal regulation of the heart occurs largely via the β_1 receptors, though β_2 and α receptors play a minor role. In the heart stimulation of β receptors leads to a positive chronotropic and inotropic effect. Blockade of these receptors would lead to a decrease in heart rate and force of contraction. In addition to this, stimulation of the M_2 receptors would lead to a decrease in heart rate and atrial contractility; while a blockade of the M_2 mediated vagal tone would lead to an increase in rate and force of contractions in the heart.

Parasympathetic nervous system exerts its control of cardiac function via the vagus nerve. Stimulation of the vagus nerve has an inhibitory effect on the cardiac pacemaker, the atrial myocardium, and atrioventricular (AV) conduction tissue, resulting in a decrease in sinus rate, atrial contractility and conduction through the AV node. There is also a lesser reduction in the force of ventricular contraction, and the net effect is a decrease in heart rate. Acetylcholine is the neurotransmitter in the parasympathetic system, and drugs, which inhibit its secretion from the vagus nerve endings, would cause an increase in heart rate.

The postganglionic sympathetic nerve fibres innervate the SA and AV nodes, the coronary vessels, the atria, and ventricular myocardium. Sympathetic stimulation increases both the rate and force of contraction of the atria and ventricles, increases sinus rate and conduction through the AV node. The net effect is an increase in the rate and force of contraction of the heart. Sympathetic stimulation could increase the heart rate to 250 b.p.m in humans in response to intense stimulation. Sympathetic stimulation of the ventricular myocardium plays a significant role in regulation of its contraction force during resting conditions. In addition to its effect on heart rate and force of contraction,

sympathetic stimulation also affects vascular resistance in the arteries. Noradrenaline, which is the postganglionic sympathetic neurotransmitter, exerts its effects on heart rate and force of contraction by acting on the β_1-adrenergic receptors on the myocardial cell surface, while it causes vasoconstriction of the arteries via the α_1 receptors in the arteries. The hormones – adrenaline and noradrenaline released from the adrenal medulla of the adrenal glands, also regulate heart function. Both hormones increase the rate and force of contraction of the heart. Secretion of these hormones from the adrenal medulla is controlled by sympathetic stimulation, and many stimuli, which increase the sympathetic stimulation of the heart, also increase the release of adrenaline and noradrenaline from the adrenal medulla.

Apart from the above factors, other factors exist in the body, which help to maintain homeostasis by the regulation of cardiac function. These however would be discussed in the next section, with reference to their significance to the experimental models used.

Other regulatory mechanisms

Sensory receptors of the baroreceptor reflex, which are sensitive to stretch, are located in walls of certain large arteries such as the internal carotid arteries and the aorta. These receptors monitor the blood pressure and respond to stretching of the blood vessels by causing changes in the rate and force of contraction of the heart. The effects of the baroreceptor reflex are only relevant in *in vivo* preparations, they are obviously non–existent in *in vitro* preparations were the large arteries are absent.

Chemoreceptors sensitive to changes in pH and carbon dioxide levels in the blood are found within the medulla oblongata. In addition to these, chemoreceptors sensitive to changes in the levels of oxygen in the blood are found in the carotid and aortic bodies. A drop in the pH of the blood or a rise in the carbon dioxide content causes a decrease in parasympathetic and an increase in sympathetic stimulation of the heart. This results in an increase in the rate and force of contraction of the heart. The resulting increase in cardiac output causes an increase in blood flow through the lungs where carbon dioxide is eliminated from the body and oxygen is introduced into the blood. The reduction of carbon dioxide in the blood helps increase the pH of the blood back to its normal values. A dramatic reduction in blood oxygen levels, such as in asphyxiation activates the carotid and aortic chemoreceptors. This leads to decrease in heart rate and an increase in

vasoconstriction. The slowing of the heart rate protects the heart for a while by reducing its oxygen demand, while the vasoconstriction helps maintain the flow of blood to the tissues in the face of a decreased heart rate.

The chemoreceptors do not normally function independent of other regulatory mechanisms, and are only relevant in *in vivo* experiments. In *in-vitro* experiments the chemoreceptors are not there to help maintain a balance in carbon dioxide and oxygen levels and pH, hence the perfusion fluid is buffered to maintain the pH within normal physiological range. A gaseous mixture of 95% O_2 and 5% CO_2 is bubbled into the perfusion fluid throughout the experiment to provide oxygen to the heart, maintain oxygen – carbon dioxide balance and help maintain pH.

2.1.1.4 Properties of the heart

The heart contracts rhythmically to pump blood through the circulatory system. The amount of blood pumped out at a particular time is determined by the rate at which the heart contracts, as well as the force with which it contracts. These properties of rate and force of contraction are known as chronotropism and inotropism.

Chronotropism

Chronotropism refers to the rate at which the heart contracts. The heart is an autorhythmic pump, whose rhythmicity can be affected by external influences. The SA node, AV node and ventricular myocardium are innervated by sympathetic and parasympathetic nerves which affect heart rate. Stimulation of the parasympathetic nerves (vagus nerve), leads to a slowing of the heart rate, a negative chronotropic effect, while stimulation of the sympathetic nerves would lead to an increase in heart rate, a positive chronotropic effect.

Inotropism

To pump blood out of the heart and into circulation, the heart has to develop enough intraventricular pressure to overcome aortic pressure. This is achieved by the contraction of ventricular muscle fibers. Inotropism refers to the force produced by the contracting ventricular muscle fibers. Inotropism is determined by different factors, the most important being the length of ventricular muscle fibers at the end of ventricular filling –

the Frank-Starling law of the heart. Parasympathetic and sympathetic stimulation also affect ventricular contraction, though parasympathetic stimulation affects it to a lesser degree than sympathetic stimulation. Sympathetic stimulation increased the force of contraction of ventricular myocardium – a positive inotropic effect, while parasympathetic stimulation decreases the force of myocardial contraction – a negative inotropic effect.

2.1.2. THE CIRCULATORY SYSTEM

The main function of the cardiovascular system is to provide nutrients and oxygen needed for metabolism by the cells of the body, and remove the toxic waste products of this metabolism. The heart pumps blood around all the tissues of the body, while the circulatory system serves as a conduit for the blood.

2.1.2.1. Description

The circulatory system is made up of the peripheral circulatory system and the coronary circulatory system. The peripheral circulatory system supplies blood pumped from the heart to the rest of the body. Beginning from the large arteries such as the aorta, the system divides and sub-divides to smaller and smaller branches until the smallest capillaries, which lie adjacent to individual cells. Blood is collected from capillaries to venules to veins up to the largest veins, the jugular veins, which channel blood back into the heart. The coronary circulation supplies the heart itself with blood.

2.1.2.2 Peripheral circulation

The heart pumps blood from the ventricles into large elastic arteries that then branch repeatedly to form progressively smaller arteries, eventually ending up as thin walled capillaries. As arteries subdivide and get smaller and smaller, there is a gradually transition from walls containing large amounts of elastic tissue and little smooth muscle to walls with little elastic tissue and a large amount of smooth muscle.

Blood flows from the smallest arteries – arterioles into capillaries where most of the exchange between the contents of blood and interstitial spaces occurs. Capillaries are thin walled, contain no elastic tissue and account for more peripheral circulation tissue than any other type of blood vessel. From capillaries, blood flows into the venous system. Veins have thinner walls, with less elastic tissue and fewer smooth muscle cells than

arteries. As they project towards the heart, the veins join up from smaller, thinner walled, numerous venules to bigger, thicker walled, fewer veins.

Blood flowing through blood vessels exerts a force against the constraining vessel walls known as blood pressure (BP). The force exerted depends on both the amount of blood flowing through the blood vessel (cardiac output - CO) and the constraints by the blood vessel walls (peripheral vascular resistance - PVR) in the following relationship.

$$BP \propto CO \times PVR \quad (2.1)$$

Peripheral vascular resistance (PVR) plays a huge role in determining blood pressure, especially in the elderly where hypertension is mostly due to a loss in elasticity, and a narrowing of the arteries. Blood pressure is measured in millimetres of mercury (mmHg), with systolic pressure indicating the blood pressure during systole and diastolic pressure indicating the pressure during diastole.

Cardiac output (CO) is the volume of blood pumped by the heart per unit of time (Litres/minute) into circulation. Cardiac output is dependent on the rate at which the heart pumps (heart rate - HR), and the volume of blood pumped out at each contraction of the ventricles (stroke volume - SV). The relationship is expressed thus:

$$CO \propto SV \times HR \quad (2.2)$$

As such, blood pressure is influenced by not only the vascular resistance exerted by arteries, but also by stroke volume and heart rate. Changes in blood pressure reflect changes in one or more of these parameters.

2.1.2.3. Coronary circulation

Cardiac muscle cells require a supply of oxygen and energy for the contraction that drives the pumping action of the heart. A network of blood vessels in the cardiac myocardium supplies this energy and oxygen requirement. Left and right coronary arteries arise from the aorta just above the point where it leaves the heart and lie within the coronary sulcus separating the atria from the ventricles. A branch of the left coronary artery, called the anterior interventricular artery, supplies the anterior part of the heart. The lateral wall of

the left ventricle is supplied by another branch of the left coronary artery called the left marginal artery, while the circumflex artery, a branch of the left coronary artery supplies the posterior side of the heart. The lateral wall of the right ventricle is supplied by a branch of the right coronary artery called the right marginal artery, while another branch of the right coronary artery, the posterior interventricular artery supplies the posterior and inferior part of the heart. The great cardiac vein drains the left side of the heart, and a small cardiac vein drains the right margin of the heart. The veins converge towards the posterior part of the coronary sulcus and empty into the coronary sinus. Coronary perfusion occurs mostly during diastole, especially in deeper parts of the myocardium, due to an increased pressure gradient from the epicardium towards the endocardium during ventricular systole.

2.1.2.4 Regulation

Nervous control of arterial blood pressure is a very important aspect of regulation of peripheral circulation. Vascular muscle tone is maintained by adrenoreceptors α_1 and β_2 located in the smooth muscles of the arteries. The stimulation of the α receptors either by sympathetic stimulation, intrinsic adrenaline or α_1 agonist agents would lead to the contraction of the vascular smooth muscles and an increase in arterial resistance, and an increase in blood pressure. Stimulation of the β_2 receptor would promote smooth muscle relaxation. Alpha blockade by α_1 receptor blockers would also lead to the relaxation of the vascular smooth muscles resulting in a drop in blood pressure. Vascular smooth muscles also respond to changes in tissue PO_2, PCO_2, and pH. A reduced oxygen concentration, increased carbon dioxide concentration and reduced pH would result in vasodilatation while changes in the opposite direction for these individual parameters would lead to vasoconstriction (Mellander, S., Johansson, B. 1968).

Coronary blood flow is subject to the same regulatory mechanisms as are other vascular beds, coronary vessels respond to direct autonomic stimuli like other blood vessels, but the major determinant of coronary resistance is cardiac oxygen consumption. Cardiac oxygen consumption in turn depends on factors such as systolic wall tension, contractile state of the myocardium, and heart rate. An increase in Inotropism in the heart would increase the oxygen demand and so lead to a dilatation of coronary arteries and increased coronary flow. Decreased Inotropism would lead to the opposite effect.

2.2 DRUGS WITH CARDIOVASCULAR EFFECTS

Apart from the internal regulatory mechanisms discussed above, the functioning of the heart can be regulated, or changed by certain drug substances. These drugs bring about their effects via different mechanisms, and act at different parts of the cardiovascular system. In the following sections, we would be looking at inotropic, chronotropic and vasoactive agents.

Inotropic agents

Inotropic agents are those agents, which alter the contractility of the myocardium. Though they act by various mechanisms, the net effect is either increase or a decrease in the force of contraction of the myocardium and stroke volume. Positive inotropic agents cause an increase in myocardial contractility, and examples include adrenaline, noradrenaline, dobutamine, dopamine and isoproterenol. Negative inotropic agents cause a decrease in the force of contraction of the myocardium, and a decrease in stroke volume. Examples of these agents include Calcium channel blockers, and the anticholinergic drug – atropine, propranolol and atenolol.

Chronotropic agents

Agents that increase or decrease the rate of contraction of the myocardium are known as chronotropic agents. As with inotropic agents, these agents could exhibit either a positive or a negative chronotropic effect on the myocardium. Positive chronotropic agents, which include adrenaline and noradrenaline, would increase the rate of contraction. Other positive chronotropic agents include Isoproterenol and dobutamine. Negative chronotropic agents would cause a decrease in heart rate; examples include β- blockers and some Ca channel blockers. A few drugs acting directly on the heart posses both inotropic and chronotropic effects.

Vasoactive agents

Vasoactive agents cause the constriction or the dilatation of blood vessels. The net effect on the heart differs depending on which vessels are constricted or dilated. Dilatation of the veins would lead to a pooling of blood in the larger veins, leading to a decrease in preload. A significant decrease in preload would lead to a decrease in force of contraction (because of a decrease in the stretching of the ventricular walls), stroke volume and cardiac output. Constriction of the veins would lead to an increase in blood flow to the

heart, leading to an increase in preload. The increase in preload leads to an increase in force of contraction, stroke volume and cardiac output. Constriction of the arteries by agents such as phenylephrine and methoxamine would lead to an increase in blood pressure as well as an increase in afterload. The increase in blood pressure would lead to a decrease in heart rate via negative feedback of the baroreceptor reflex. The increase in afterload also leads to a decrease in heart rate, though its effect is minimal. Dilatation on the other hand by prazosin and phenoxybenzamine would decrease blood pressure as well as afterload. The decrease in blood pressure leads to reflex tachychardia via the baroreceptor reflex. Constriction of the coronary vessels would reduce blood flow to the myocardium, decreasing the amount of Oxygen and energy available the cells. This would adversely affect cardiac function. Dilatation of the coronary vessels would increase blood flow to the myocardium and so improve cardiac function.

2.3 ANIMAL MODELS FOR EVALUATING CARDIOVASCULAR ACTIVITY

Various animal models exist for the evaluation of cardiovascular activity of plant extracts. These are broadly classified into *in vivo* and *in vitro* methods. *In vivo* methods involve the use of whole animals as test subjects, and results obtained are representative of the action of the extract on humans. Examples of animal models include; the anaesthetized normotensive rat, spontaneously hypertensive rats, 2-Kidney 1-clip hypertensive model, DOCA/salt hypertensive model and salt-loaded hypertensive model. Normotensive rat models are ideal for pharmacological screening when the action of the test substance cannot be predicted, while the hypertensive models are used to assay antihypertensive effect. *In vitro* methods on the other hand involve the use of isolated organs or parts of organs in simulated physiological environment. Examples of preparations of parts of organs include rabbit ear artery, rat mesenteric artery, and rat or rabbit aorta preparations, while an example of the isolated perfused organ preparation for *in vitro* experiments is the isolated perfused heart (langendorff and working heart) model. *In vitro* methods are generally used to study the effects of plant extracts on individual organs or parts of organs and give a clearer idea of the actions of the test substances. For the present work the anaesthetized normotensive rat model was used for *in vivo* experiments and the double-sided working heart model used for *in vitro* experiments.

2.3.1. THE ANAESTHETISED NORMOTENSIVE RAT MODEL

The anaesthetized normotensive rat model allows the researcher to record changes in blood pressure and heart rate in intact animals, in response to administered drugs. The rat is anaesthetized with sodium pentobarbitone, injected intra-peritoneal, and the trachea exposed and cannulated for artificial respiration. To record changes in heart rate and blood pressure, the carotid artery, the abdominal aorta or the femoral artery is exposed and cannulated. The cannula is connected to a pressure transducer and thence to a suitable instrument to record changes in blood pressure and heart rate. Drug substances are infused via a cannula inserted into either the jugular or femoral vein. One of the advantages of the anaesthetized heart model is that suitably prepared and kept under anaesthesia, the experiment could run for more than 24 hours. The anaesthetized normotensive rat model records changes in heart rate, systolic pressure, diastolic pressure and mean arterial pressure. Normal ranges are between 116mmHg – 145mmHg (systolic), 76mmHg – 97mmHg (diastolic), 103mmHg – 129mmHg (mean arterial pressure) and 296 – 388pbm (heart rate) for a 300g rat (Livius et al 2000). Male animals are preferred to female animals, and young rats are used instead of old ones. This is because in the older rats, the responsiveness of the heart to stimulation is reduced, the walls of the arteries are less elastic and as with humans, older rats tend to be spontaneously hypertensive

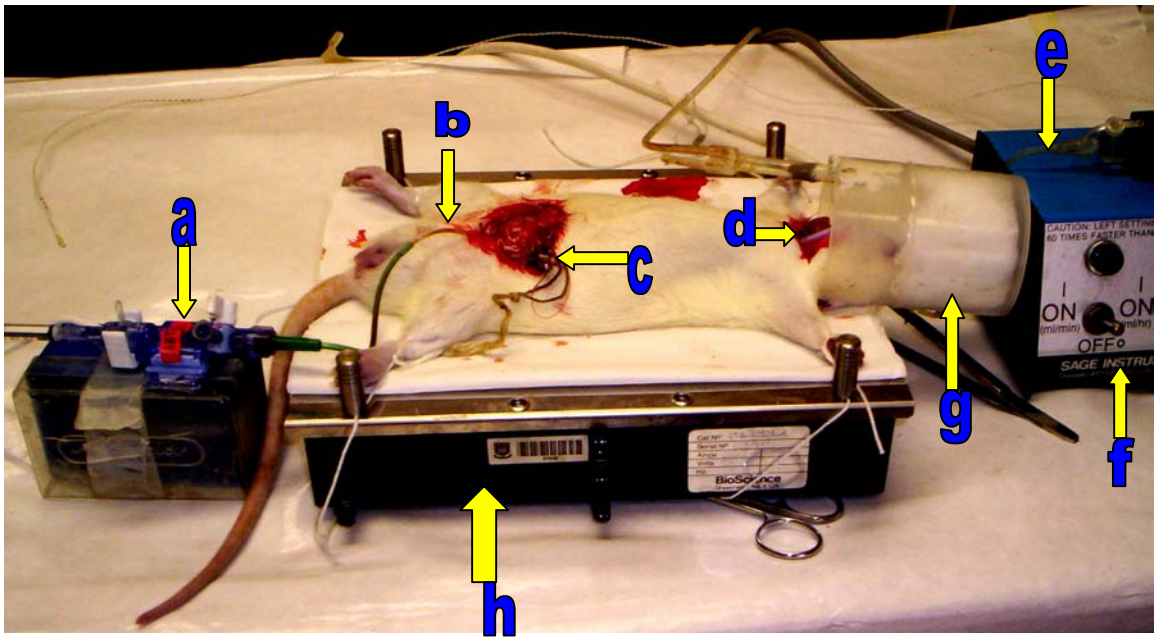

Figure 2.2: The anaesthetized normotensive rat model (a= BP transducer; b= arterial catheter; c= bulldog clamp; d= trachea tube; e= venous catheter; f= syringe pump; g= Oxygen mask; h=small animal operating table).

2.3.2. ISOLATED PERFUSED HEART MODELS

Of the different types of *in vitro* cardiovascular models available, the isolated perfused small mammalian heart has been recognized as a suitable experimental model for studying many physiological, biochemical and toxicological aspects of cardiac function. This model also most closely represents the best compromise between the quantity and quality of data that can be obtained from an experimental model as against the relevance of such results to human populations (Sutherland, F., and Hearse, J. 1999). Several preparations of the isolated perfused small mammalian heart are currently in use, and these include the retrograde perfusion system, developed originally by Langendorff, and the working heart model developed by Neely and Taegtmeyer (Depre, C. 1998). Under resting conditions, there are normally no dramatic changes in the temperature of the heart and its surroundings, although such changes if they occur would affect the heart rate. Small increases in the myocardial temperature would cause an increase in heart rate, while a decrease in temperature has the opposite effect (Don Stevens, E. et al 1972). In isolated perfused heart systems, small changes in temperature would lead to large variations in cardiac function. Keeping the temperature of the preparation within normal limits becomes very important. The effect of temperature on heart rate is exploited in *in vitro* preparations. The excised heart is placed in very cold fluid to stop the contractions of the heart completely and prevent ischemic injury.

2.3.2.1 Langendorff model

The Langendorff model was the first model described for the perfusion of isolated hearts. This model involves the retrograde perfusion of isolated hearts, and though it enables the study of a functioning heart, the interpretation of the results and their projection to *in vivo* situations is limited. In this model, animal hearts, mostly from rats, rabbits or ferrets are rapidly excised and immersed in ice-cold perfusion fluid to stop contractions. The aorta is then cannulated and the heart perfused via the aorta (retrograde perfusion) at a constant hydrostatic pressure from a column or at a constant flow using flow-regulating pumps. In the Langendorff model, a latex balloon can also be inserted into the left ventricle and inflated to induce a left ventricular end-diastolic pressure. Left ventricular pressure, heart rate, developed pressure and coronary perfusion pressure can be recorded from this model (Depre, C. 1998).

2.3.2.2. Working Heart model

The circulatory system of mammals is similar across species and so small mammalian heart preparations can be used to study metabolic processes and the effects of drugs on these processes, the results of which can be extrapolated to humans. The working heart model is set up in such a way as to mimic the normal body circulation. In this model, hearts from anaesthetized animals, preferably rats, are rapidly excised, and dropped into ice-cold perfusion fluid to stop the heart beating. The aorta is the cannulated and then perfused retrogradely in the langendorff mode at constant hydrostatic pressure (100mmHg for a 300g rat) to remove all blood cells from the organ. Meanwhile, the left atrium is cannulated, and then after a sufficient period of langendorff perfusion, the heart is perfused in "working" conditions. The heart in "working" conditions is perfused antegradely with the perfusion buffer entering the heart via the left atrium, into the left ventricle and then ejected through the aortic root against an afterload column to recirculate through the heating and aerating sections, back into the left atrium (Depre, C. 1998). In langendorff perfusion, the perfusion fluid enters the ventricles through the cannulated aortic root. The afterload is set to simulate the normal pressure in rat aorta of 116-145mmHg (for a 300g rat) by a bubble trap set at 100cmH_20 (above the heart) and connected to the aortic cannula (Livius, V., *et al* 2000). To simulate a pre load of 76-97mmHg (for a 300g rat), the left atrial cannula is connected to a bubble trap set at 15cmH_2O (Livius, V., *et al* 2000).

Composition of perfusion fluids used in isolated perfused heart experiments vary, but those used in most studies are based on the Krebs-Hanseleit perfusion fluid model (Sutherland, F., Hearse, J. 1999). This fluid which is supposed to mimic both the key ionic composition and the pH of blood has the following composition: Sodium chloride (NaCl) 118.5mM, Sodium bicarbonate ($NaHCO_3$) 25.0mM, Potassium Chloride (KCl) 4.7mM, Magnesium Sulphate ($MgSO_4$) 1.2mM, Potassium Di-hydrogen Phosphate (KH_2PO_4) 1.2mM, and Calcium Chloride ($CaCl_2$) 2.5mM. The pH of the buffer solution at optimal temperature of 37.0^oC is 7.4, and the energy requirement of the working heart is supplied by adding glucose. The problems of precipitating calcium and phosphate ions is reduced by lowering the pH by gassing the solution with 95%O_2 + 5%CO_2 before adding calcium chloride.

2.3.2.3. Double-sided working heart model

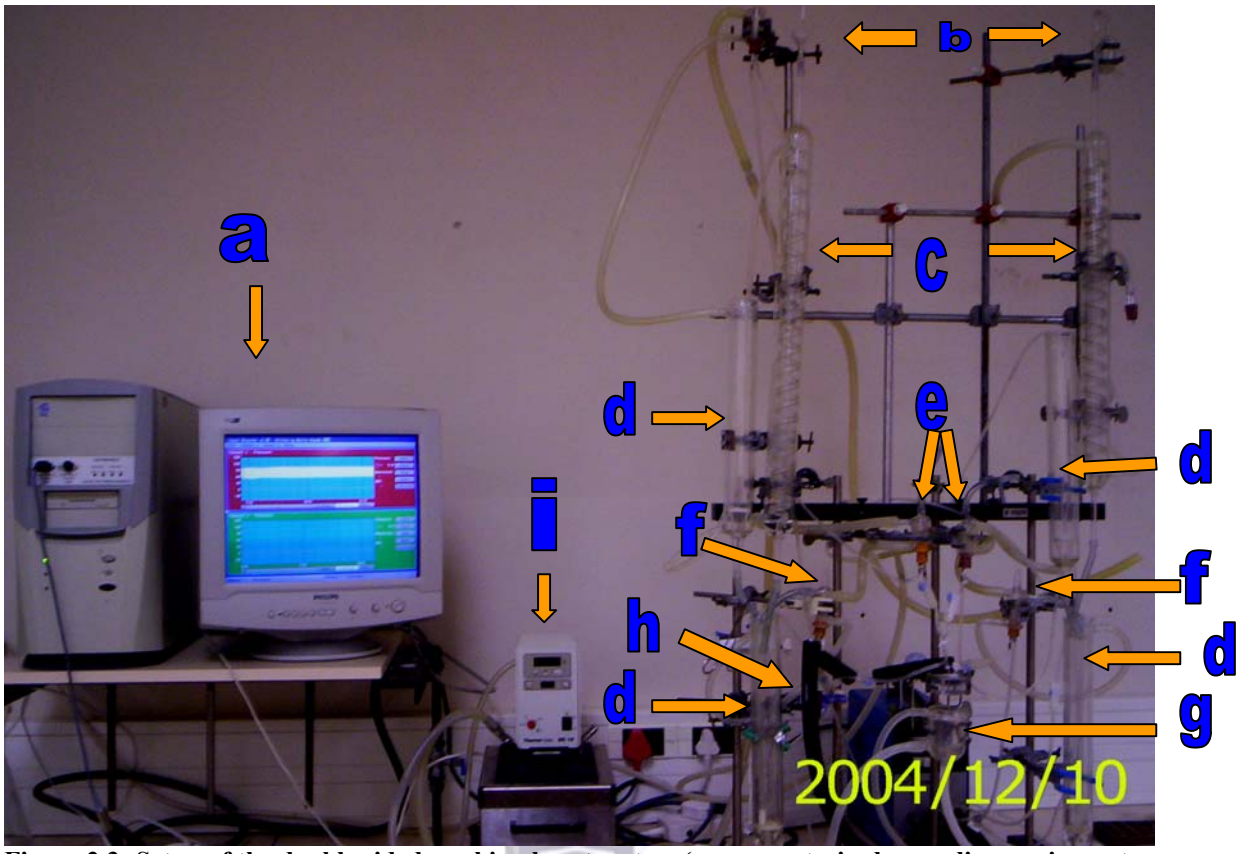

Figure 2.3: Setup of the double sided working heart system (a= computerised recording equipment; b= cardiac output compliance chamber; c= cardiac output collector; d= reservoir; e= aortic compliance chamber; f= pulmonary vein bubble trap; g= organ chamber; h= BP transducer; i= water bath and circulator.

The double-sided working heart system is a modification of the working heart system described previously by Njagi, that allows for the perfusion of the heart with two different perfusion fluids that do not mix (Njagi, A. 2004). It actually consists of two working heart models placed side by side and connected to one aortic cannula and one atrial cannula. A system of taps allows for the switching of perfusion from langendorff to working heart, within each of the two working heart models, and also for switching from one working heart model to the other (Njagi, A. 2004). On the double-sided working heart model, the systolic pressure (SP), diastolic pressure (DP), developed pressure (dp), cardiac output (CO), aortic output (Qa) and coronary flow (Qe) are measured. In the working heart system, the aortic pressure is measured as opposed to the left ventricular pressure. The aortic output measured is the amount of fluid pumped by the left ventricle per minute against the 100cm H_2O, and it is used as a measure of how efficiently the heart is working. Coronary flow is measured as the effusate from the organ chamber per minute during both retrograde and antegrade perfusion. Coronary flow is affected by the rate of contraction of the heart, since coronary circulation is minimal when the

myocardium contracts, blood clots and air bubbles. The double-sided working heart model contains a system of bubble traps to prevent air bubbles from entering the coronary circulation.

CHAPTER THREE

MATERIALS AND METHODS

This chapter describes and lists the materials and chemicals used in the study. The different methods used for the study are also explained in detail here.

3.1 MATERIALS

3.1.1 CHEMICALS AND DRUGS

All chemicals used in the study were of standard grade.

I) Chemicals used in the extraction of plant material

a) Hexane (Kimix)
b) Ethyl acetate (Kimix)
c) Silica gel (35-60 mesh and 70-230 mesh) (Aldrich)
d) Methanol (Aldrich)
e) Acetone (Merck)
f) Distilled water

II) Chemicals and drugs used in the anaesthetized normotensive rat model

a) Chemicals
   i) Sodium pentobarbitone (Kyrn Laboratories)
   ii) Diethyl ether (Kimix)
   iii) Dimethylsulfoxide (Sigma)
   iv) Heparin (Bodene)
   v) Tween 80 (Sigma)
   vi) Oxygen (Afrox)
b) Experimental drugs
   i) Adrenaline (Bodene)
   ii) Prazosin (Sigma)
   iii) Atenolol (Zeneca)
c) *Leonotis leonurus.* Crude aqueous extract
d) *Leonotis leonurus.* Fraction C of methanol extracts

III) Chemicals and drugs used in the double-sided working heart model

a) The Krebs-Hanseleit buffer solution with the following composition (in mmol):

i) Sodium chloride (NaCl) 119.00

ii) Sodium bicarbonate ($NaHCO_3$) 25.00;

iii) Potassium chloride (KCl) 4.75;

iv) Di-hydrogen potassium phosphate (KH_2PO_4) 1.2;

v) Magnesium Sulphate ($MgSO_4.7H_2O$) 0.6;

vi) Sodium Sulphate (Na_2SO_4) 0.6;

vii) Calcium chloride ($CaCl_2.H_2O$) 1.25

viii) Glucose 10.00.

All the above chemicals were obtained from Merck chemicals

b) Carbogen (95%Oxygen, 5% Carbon dioxide) (Afrox Pty Ltd)

c) Drugs

i) Adrenaline (Bodene)

ii) Atenolol (Zeneca)

d) *Leonotis leonurus.* Fraction C of methanol extracts

3.1.2 EQUIPMENT

I) Equipment used in extraction of plant material

a) Soxlet

b) Grinder

c) TLC aluminum sheets (Silica gel 60 F_{254}; 20 X 20cm) (Merck)

d) Chromatography Column

e) Rotovapour

II) Equipment used in the double-sided working heart model

a) Working heart system glassware

i) Air traps

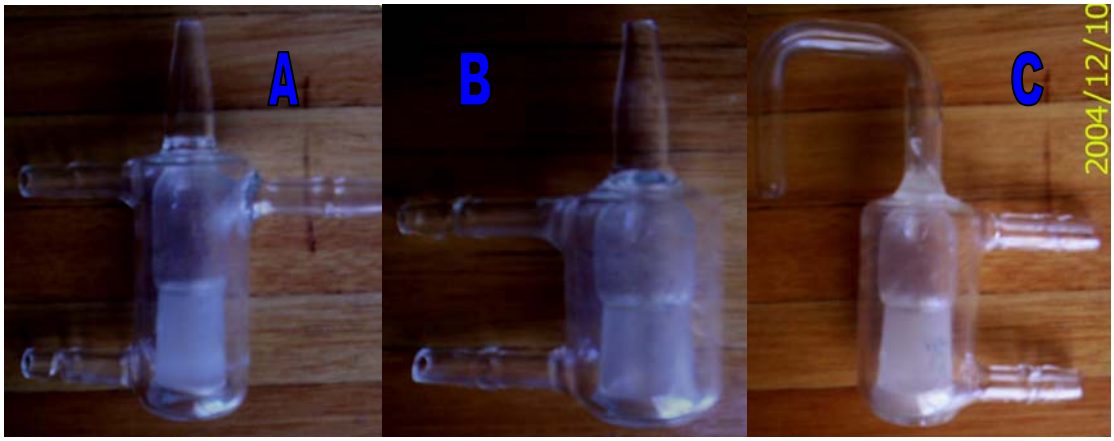

Figure 3.1: Compliance chambers and bubble traps (A= Aortic compliance chamber; B= Pulmonary vein bubble trap; C= Cardiac output compliance chamber).

ii) Glass condensers (types A and B)

Figure 3.2: Glass condensers: A serves a reservoir for perfusion fluid and is jacketed to keep perfusion fluid at constant temperature. B is used to capture the cardiac output and connects to the reservoir.

iii) The organ chamber

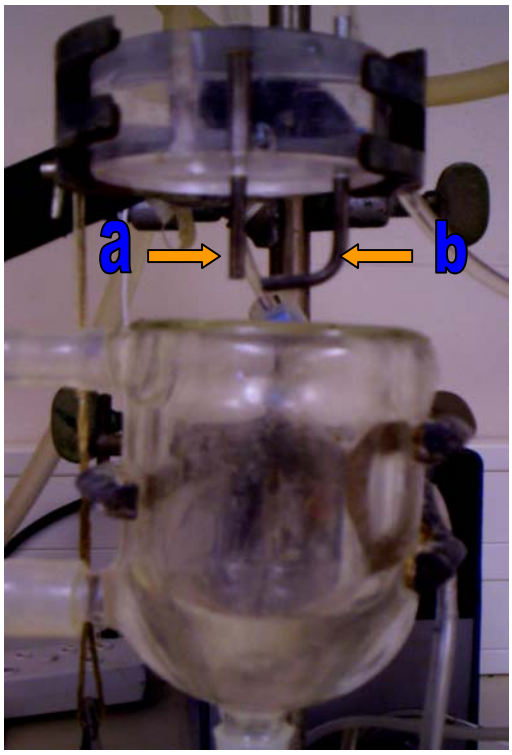

Figure 3.3: Organ chamber: (a= aortic cannula; b= pulmonary cannula).

b) Chart recorder V2 (Genotronics)

c) Chart recorder v. 2.0 software

d) Blood pressure amplifier

e) Synchronised double pump (Masterflex)

f) Circulator + water bath - Thermo Haarke B3 (Lab and scientific equipment)

III) Equipment used in the anaesthetized normotensive rat model

a) Small animal operating table (BioScience)

b) Blood pressure transducer (AD instruments)

c) Power Lab 4/20T (AD instruments)

d) Chart 4.0 software (AD instruments)

e) Syringe pump (sage instruments)

f) Oxygen mask

3.1.4 ANIMALS

Animals used in the study were male Wistar rats between the age range of 3-4months. Rats were used because they provided the best compromise between size and heart rate, as against rabbits which have heart rate that are quite high. Only male wistar rats were

used, though some researchers had indicated that there were no gender specific differences in cardiovascular parameters, to ensure uniformity. The age range of 3-4 months was chosen because that was the optimal age for male Wistar rats. Older rats have lower heart rates and have a reduced sensitivity to cardioactive substances, probably due to loss of elasticity in the arteries with age (Livius et al 2000).

3.2 METHODS

The plant was collected from Montague gardens in South Africa, while the animals were obtained from the Medical Research Council (MRC), Tygerberg and the department of physiology, University of the Western Cape (U.W.C).

3.2.1 PLANT EXTRACTION

The fresh leaves of the plant were washed in water and dried in a hot air oven at 30^{o} C for 72 hours. 734.3grams of the dry leaves was then milled to a fine powder and the powder extracted in distilled water for 48 hours using the soxhlet extractor. The aqueous extract was quickly frozen to -82 ^{o} C, and the frozen extract dried *en vacuo* for 48 hours using a freeze-dryer to yield 213.4grams of dry extract. The freeze-dried powder was then stored in sealed, amber coloured bottles at 4 ^{o} C. The freeze-dried powder was then reconstituted with normal saline during experiments to give the aqueous extract.

Methanol extracts of the powdered plant was also prepared by complete extraction of 875.6 grams of the powder, using methanol as a solvent, in a soxhlet extractor for 72 hours. Excess methanol was then evaporated using a roto-vapour to yield 375.2 grams of extract. Analytical thin layer chromatography (TLC) of the methanol extract was carried out using aluminium backed TLC sheets containing F_{254} dye and a mixture of ethyl acetate and hexane as the solvent phase in the following ratios:

i) Hexane: Ethyl acetate (1:0)
ii) Hexane: Ethyl acetate (9.5:0.5)
iii) Hexane: Ethyl acetate (9:1)
iv) Hexane: Ethyl acetate (4:1)
v) Hexane: Ethyl acetate (3:1)
vi) Hexane: Ethyl acetate (2:1)

Column chromatography was then carried out over 148 hours on the methanol extract using hexane: ethyl acetate (4:1) as mobile phase and silica gel (70-230 mesh grade) as

stationary phase. TLC was carried out on the fractions collected to determine those that contained compounds with the same RF values and these were pooled and evaporated. The resulting fractions were labelled as fractions A, B, C, D and E.

3.2.2 *IN VIVO* STUDY

The aqueous extract of *Leonotis leonurus* and fractions A, B, and C from chromatography of the methanol extract were tested on male wistar rats using the anaesthetized normotensive rat model as described in section 2.3.1 of chapter two. Only the first three fractions (A, B, and C) were tested because of time constraints.

3.2.2.1 Preparation and cannulation of animals

Healthy male wistar rats aged less than 4 months, and within the weight range of 250-400g were used for the study. The arterial and venous cannula were cleaned and filled with a 10% solution of heparin to prevent blood coagulation. The animals were weighed and anaesthetized with sodium pentobarbitone (40mg/kg) injected intra-peritoneally. Animals under anaesthesia were then transferred to a small animal operating table and secured.

The trachea was accessed via a midline incision in the anterior cervical region, a cut made into it and a lubricated tracheal tube carefully inserted and secured in place with a knot. The tracheal tube was cleared of fluids, an oxygen mask placed over the head, and the animal allowed to stabilize. The external jugular vein was then exposed, a bulldog clamp placed towards the heart to prevent blood loss. The exposed section of the jugular vein was filled with blood by gently massaging the head, and tied off towards the head to prevent the blood draining away. An incision was made on the vein, and the lubricated venous catheter carefully inserted into the vein and secured in place. An incision was made in the lower abdominal region and the femoral artery carefully separated from the accompanying vein, nerve and tissues. The artery was tied off towards the 'leg end' and a bulldog clamped on the 'abdominal end'. An incision was made in the artery and a lubricated arterial catheter inserted and secured. The incisions were then cleaned and sutured up using catgut sutures, covered with gauze and kept moist for the duration of the experiment. The animal was then allowed to rest for 30 minutes before the infusion of drug substances. During this stabilization period, the tap on the blood pressure transducer was closed towards the cannula, and the blood pressure zeroed on the chart 4 software.

The tap was then opened to the cannula, and the blood pressure recording on a computer running the chart 4 software started. A temperature probe was also inserted into the rectum to monitor the animal's temperature throughout the experiment. After the 30 minute stabilization period, only animals fulfilling the criteria; systolic pressure - 116mmHg – 145mmHg; diastolic pressure - 76mmHg – 97mmHg; mean arterial pressure - 103mmHg – 129mmHg; and heart rate - 296 – 388pbm were exposed to the test substances.

3.2.2.2 Experimental protocol

Animals were divided into 8 groups, each group receiving different test and standard drug substances.

a) Group I. Adrenaline – Dose response curve (0.001 – 0.008mg).

b) Group II. Atenolol – Dose response curve (0.1 – 10mg/kg).

c) Group III. Prazosin – Dose response curve (20 - 100µg/kg).

d) Group IV. Aqueous extract of *Leonotis leonurus* - Dose response curve (0.5 - 7.0mg).

e) Group V. Fractions of methanol extracts of the leaves of *Leonotis leonurus*.

f) Group VI. Aqueous extracts of *Leonotis leonurus* and atenolol.

g) Group VII. Aqueous extracts of *Leonotis leonurus* and prazosin.

Table 3.1 shows a sample of the randomized dosing for the aqueous extract of *Leonotis leonurus.*

Table 3.1: Randomized dosing for the aqueous crude extract of *Leonotis leonurus.*

Pump Position	1	1	2	1	1	1	1	1
Drug Dose mg)	4	1	6	3	7	2	5	0.5
Volume Given	0.4ml	0.1ml	0.4ml	0.3ml	0.07ml	0.2ml	0.5ml	0.05ml
Time	4mins	1min	4mins	3mins	42secs	2mins	5mins	30secs

Table 3.2 shows the protocol followed in infusing the test and standard substances in group I – V animals.

Table 3.2: Drug dosing for group I to group V animals.

Stabilize Animal 30mins	Control Normal Saline (0.3ml)	Drug dose 1 (Random Dosing)	Saline Flush 0.05ml	Stabilize	Drug dose 2 (Random Dosing)	Saline Flush 0.05ml	Stabilize

Table 3.3 shows the protocol followed in infusing standard drug (atenolol – 2mg) and test substance (aqueous extract of *Leonotis leonurus*).

Table 3.3: Drug dosing for atenolol and crude aqueous extract of *Leonotis leonurus*.

Stab. animal 30mins	Control Normal Saline (0.3ml)	*L. l* (R. D)	Saline Flush 0.05 ml	Stab. 10mins	Atenolol 2mg	Saline Flush 0.05ml	Stab. 10mins	*L. l* (R. D)	Saline Flush 0.05 ml	Stab.

Stab = stabilize; *L .l = Leonotis leonurus*

Table 3.4 shows the protocol followed in infusing standard drug (prazosin – 60µg/kg) and test substance (aqueous extract of *Leonotis leonurus*).

Table 3.4: Drug dosing for Prazosin (60µg/kg) and crude aqueous extract of *Leonotis leonurus*.

Stab. animal 30mins	Control Normal Saline (0.3ml)	*L. l* (R. D)	Saline Flush 0.05 ml	Stab. 10mins	Prazosin 60µg/kg	Saline Flush 0.05ml	Stab. 10mins	*L. l* (R. D)	Saline Flush 0.05 ml	Stab.

Stab = stabilize; *L .l = Leonotis leonurus*

3.2.2.3 Parameters assessed

For all the drugs used, the parameters that were assessed included heart rate (HR), systolic pressure (SP), diastolic pressure (DP) and mean arterial pressure (MAP). The effects of plant extracts and the standard drugs on these parameters were evaluated.

3.2.3 *IN VITRO* STUDY

The effects of fraction C of the methanol extracts, adrenaline and atenolol on the isolated perfused heart were evaluated using double sided working heart system described in section 2.3.2.3 of chapter two.

3.2.3.1 Preparation of animals

Animals used were male wistar rats weighing between 250-350g and less than 4 month old. The animals were anaesthetized using sodium pentobarbitone administered by intra-peritoneal injection. A trans abdominal incision was made to access the diaphragm. A bilateral incision was made along the lower margin of the last rib, and diaphragm carefully cut to expose the thoracic cavity. The cut out section of the thoracic cage was reflected over the animals' head to expose the heart, and the heart quickly excised (less than 30 seconds after exposing the thoracic cavity), and immediately immersed in cold perfusion fluid (<4^{o}C) to reduce the risk of ischemia.

3.2.3.2 Cannulation and perfusion

The excised heart was cleaned of excess tissue and the aorta eased onto the aortic cannula and secured in place using a thread. Retrograde perfusion (100cm H_2O pressure) of Krebs-Hanseleit buffer solution was then started. A small incision was made at the base of the coronary artery to facilitate draining. Either of the two pulmonary veins was eased onto the pulmonary cannula and secured in place using a piece of thread. A temperature probe was carefully inserted into the small incision made at the base of the coronary artery to monitor temperature of the heart.

3.2.3.3 Protocol of experiment

Animals were divided into 3 groups, each group receiving different test and standard drug substances.

a) Group I. Fraction C of the methanol extract of *Leonotis leonurus* (0.01mg/ml)
b) Group II. Adrenaline – Dose response curve (0.001 – 0.008mg/ml).
c) Group III Atenolol – Dose response curve (0.01 – 0.1mg/ml)
d) Group III. Fraction C of the methanol extract of *Leonotis leonurus* (0.01mg/ml) and atenolol (0.07mg/ml).

The following protocol was used for the administration of *Leonotis leonurus* and adrenaline.

Langendorff (pf side) 10min	Work heart (pf side) 10min	Work heart (drug side) 5min	Work heart (pf side) 10min	Work heart (drug side) 5min	Work heart (pf side) 10min

Pf= Perfusion fluid

To evaluate the effect of atenolol (0.07mg/ml) on the actions of fraction C of the methanol extract of *Leonotis leonurus* (0.01mg/ml), the following protocol was used.

Langendorff (pf side) 10min	Work heart (pf side) 10min	Work heart (drug side) – *L. leonurus* 5min	Work heart (pf side) 10min	Work heart (drug side) – *L. leonurus* & atenolol 5min	Work heart (pf side) 10min

Pf= Perfusion fluid

3.2.3.4 Parameters assessed

A pressure transducer was connected to the aortic cannula via a side arm. This transducer was connected to a computer running the Chart recorder (version 2.0) software for recording heart rate (HR), systolic pressure (SP), diastolic pressure (DP) and developed pressure (Du). A measuring cylinder was used to collect fluid running out of the organ chamber as coronary flow (Qe). Another measuring cylinder was used to collect fluid from the cardiac output compliance chamber as cardiac output (Co). Aortic flow (Qa) was also measured. Readings were taken at the beginning and at the end of perfusion of each drug.

3.3 DATA ANALYSIS

Data from experiments using the anaesthetized normotensive rat model was expressed as change in systolic pressure (SP), diastolic pressure (DP), mean arterial pressure (MAP) and heart rate (HR). This change is calculated as the difference between the value of the parameter just before the administration of the drug substance and the value at the peak of effect of the drug substance. Mean change was calculated and statistically analysed using the student's t test for significant difference ($p<0.05$).

For the working heart model, means of the values for the different parameters (Qe, Qa, CO, SP and DP, Developed pressure and HR) when the heart was perfused with perfusion fluid, fraction C of the methanol extract of *Leonotis leonurus* (0.01mg/ml), adrenaline (0.001 – 0.008mg) and a mixture containing a 0.01mg/ml solution of fraction C of the methanol extract of *Leonotis leonurus* and a 0.07mg/ml solution of atenolol was analysed for statistical significance using the Student's test ($p<0.05$). Percentage change (%$\Delta \pm$ SD) in Qe, Qa, CO, SP, DP, dp and HR when the perfusion fluid was switched Krebs-Hanseleit perfusion fluid to the drugs was calculated.

3.4 ETHICAL CONSIDERATIONS

The animals were treated according to the University of Western Cape animal regulations Act.

CHAPTER FOUR

RESULTS AND DISCUSSION

This chapter sets out the results obtained on the vascular and cardiac effects of both standard and test substances on anaesthetized rats and isolated perfused rat hearts. Tables and graphs would be used to explain the observed effects of both standard and test substances on the various parameters monitored during the study. The results stated would also be discussed.

4.2 *IN VIVO* EXPERIMENT RESULTS

This model involved the use of anaesthetised normotensive rats to study the effect of drug substances administered IV on the systolic pressure (SP), diastolic pressure (DP), mean arterial pressure (MAP) and heart rate (HR) of the animal. Fractions A, B and C from column chromatography of the methanol extracts of the plant were tested on male Wistar rats using the anaesthetized normotensive rat model. Fractions A and B killed the animals at all the doses tested (0.01mg, o.1mg, 1mg and 10mg) and so no cardiovascular data on these fractions are presented in the results. The 2mg and 60µg/kg dose of atenolol and prazosin respectively were used on group vi and vii animals (see section 3.2.2.2) because at the higher doses of these antagonists, SP, DP, MAP and HR did not return to baseline levels, even 1hour after drug administration. In fact most animals died after administration of doses higher than 2mg and 60µg/kg for atenolol and prazosin respectively. No standard drugs were co-administered with fraction C from. The following results were obtained for the different substances administered.

4.2.1 EFFECT ON BLOOD PRESSURE

4.2.1.1 Effect on Systolic pressure (SP)

Adrenaline

Figure 4.1 shows the effect of adrenaline administered in a dose range of 0.01mg to 0.08mg. Adrenaline had a dose dependent effect on the systolic pressure, with the change in pressure increasing as the dose was increased. At low doses (0.01mg), it produced a 1.6mmHg ± 0.33 increase in systolic pressure, and at the highest dose administered (0.08mg), it produced a 129.5mmHg ± 1.8 change in systolic pressure.

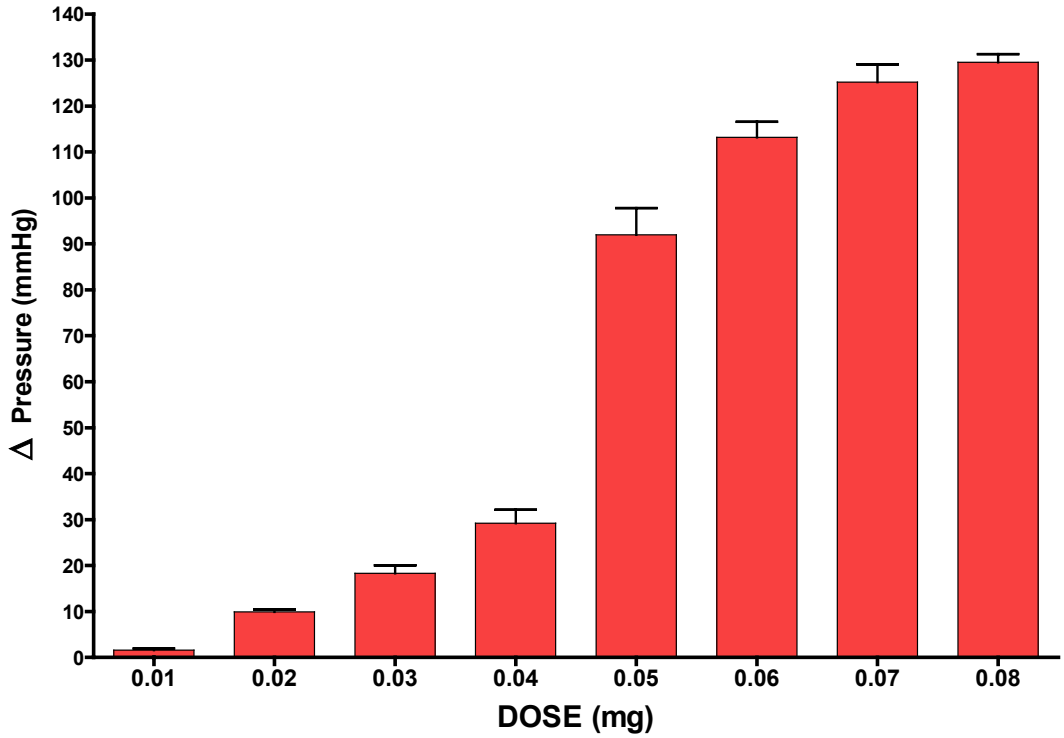

Figure 4.1: Effect of adrenaline on systolic pressure.

'The effect of adrenaline is due to a positive chronotropic and positive inotropic effect on the heart as well as a vasoconstrictive effect on the blood vessels.

Atenolol

The effect of atenolol administered in the dose range of 0.1mg to 3.0mg is shown in figure 4.2 below. As expected, atenolol produced an effect opposite to that of adrenaline on the systolic pressure, with the decrease in systolic pressure increasing with increase in dose administered. The lowest dose (0.1mg) decreased systolic pressure by -1.68mmHg ± 1.54, while the largest dose of 3.0mg decreased systolic pressure by -14.24mmHg ± 3.74.

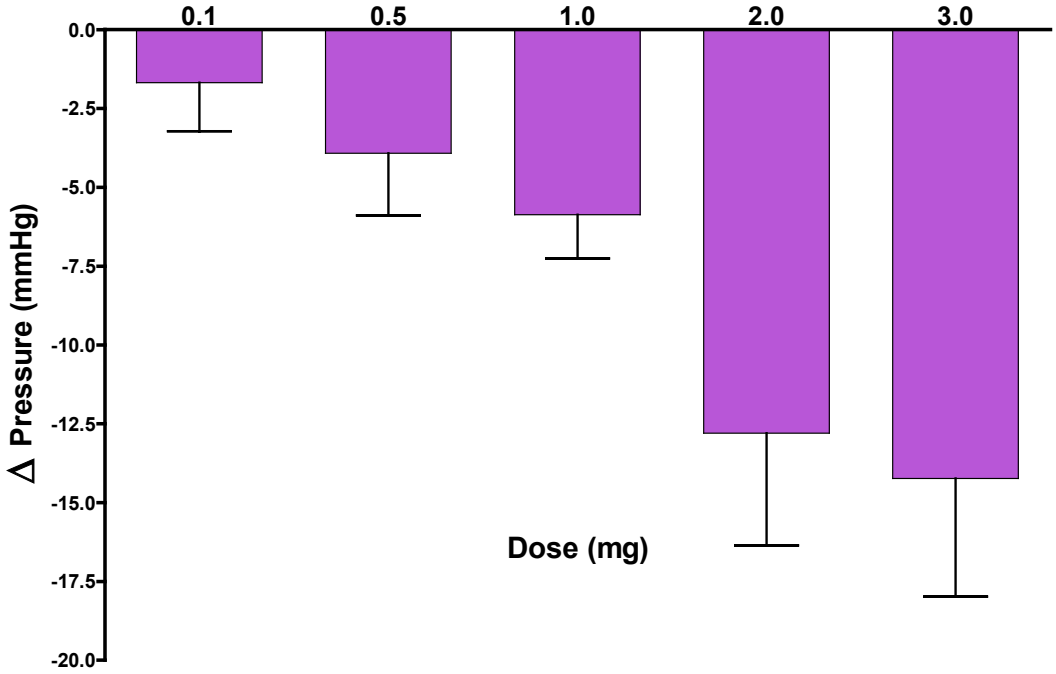

Figure 4.2: Effect of atenolol on systolic pressure.

Atenolol is a selective β_1 antagonist and its effect on blood pressure is due to its negative chronotropic and negative inotropic effect on the heart.

Prazosin

Figure 4.3 shows the effect of prazosin (20µg/kg - 100µg/kg) on systolic pressure. When prazosin was administered, it produced a decrease in systolic pressure. A 20µg/kg dose decreased systolic pressure by -8.25mmHg ± 4.52, while the highest dose (100µg/kg) decreased systolic pressure by -22.97mmHg ± 6.64.

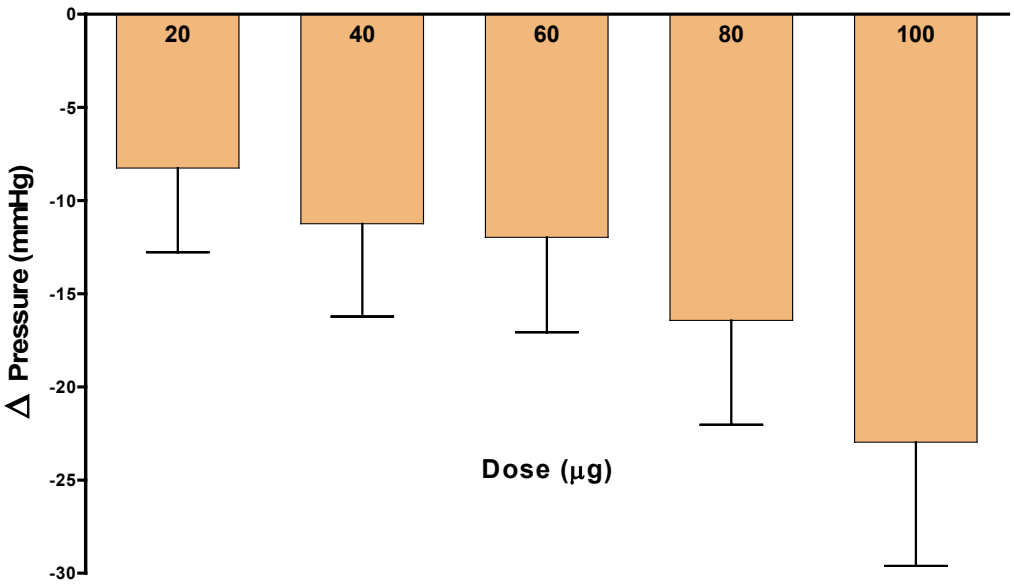

Figure 4.3: Effect of prazosin on systolic pressure.

Prazosin selectively blocks the α_1 receptors present in the blood vessels. Its blockade of this receptor leads to vasodilatation and thus a fall in vascular resistance.

Crude aqueous extract of *Leonotis leonurus*

The crude aqueous extract of *Leonotis leonurus* was administered in the dose range of 0.5mg to 7.0mg. Figure 4.4 below shows the effect of the crude aqueous extract on systolic pressure. The effect was dose dependent with the lowest dose (0.5mg) increasing systolic pressure by 4.0mmHg ± 0.18, and the highest dose (7.0mg) increasing systolic pressure by 17.0mmHg ± 0.56. The increase in systolic pressure at all doses was statistically significant (p<0.05).

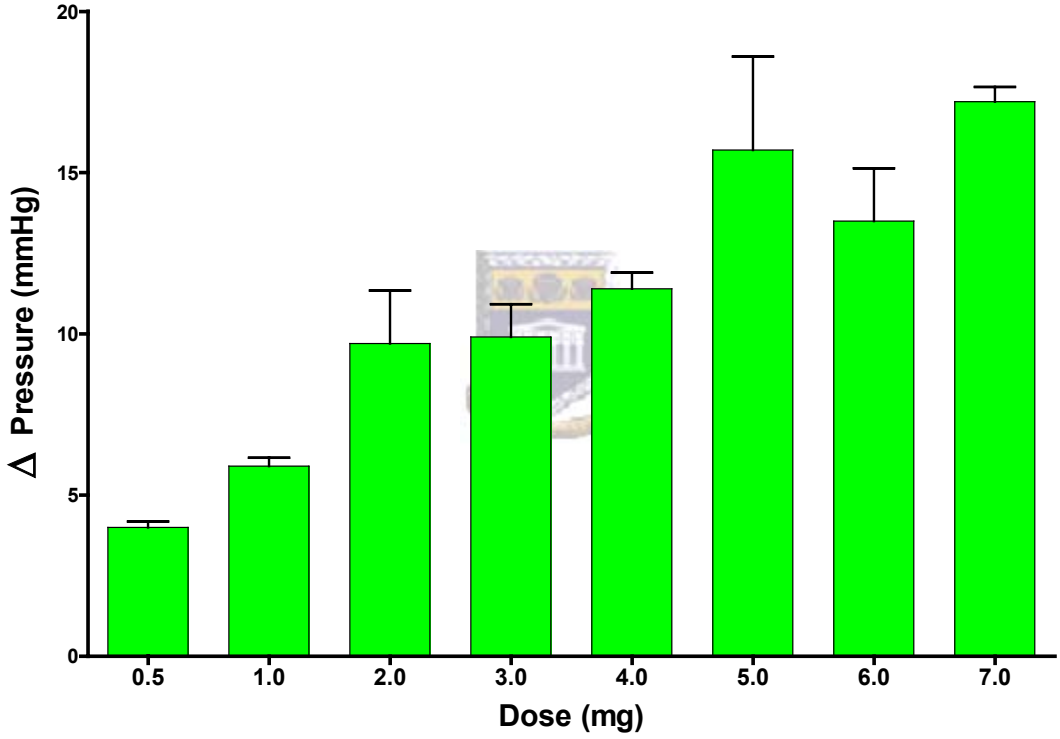

Figure 4.4: Effect of the crude aqueous extract of *Leonotis leonurus* on systolic pressure.

Crude aqueous extract of *Leonotis leonurus* and atenolol

Figure 4.5 below shows the effect of atenolol (2mg) on the cardiovascular effects of the aqueous crude extract of *Leonotis leonurus* (0.5mg – 7.0mg). Pre-administration of atenolol reduced the increase in systolic pressure for all doses of *Leonotis leonurus* used. At lower doses of *Leonotis leonurus* (1.0mg and 2.0mg), atenolol caused a great decrease in the change in pressure (from 5.90mmHg ± 0.26 to 0.78mmHg ± 1.25 and from 9.7mmHg ± 1.64 to 4.86mmHg ± 2.02 respectively). At the lowest dose of *Leonotis*

leonurus (0.5mg), there was actually a decrease in pressure with the pre-administration of atenolol. Systolic pressure decreased below the control value (0.0mmHg) by -0.25mmHg ± 1.23. At higher doses (3.0mg – 7.0mg) however, there was a lesser reduction in the change in systolic pressure by atenolol. The change in systolic pressure with atenolol was statistically significant at the low doses (0.5mg and 1.0mg; p=0.0115 and <0.0001).

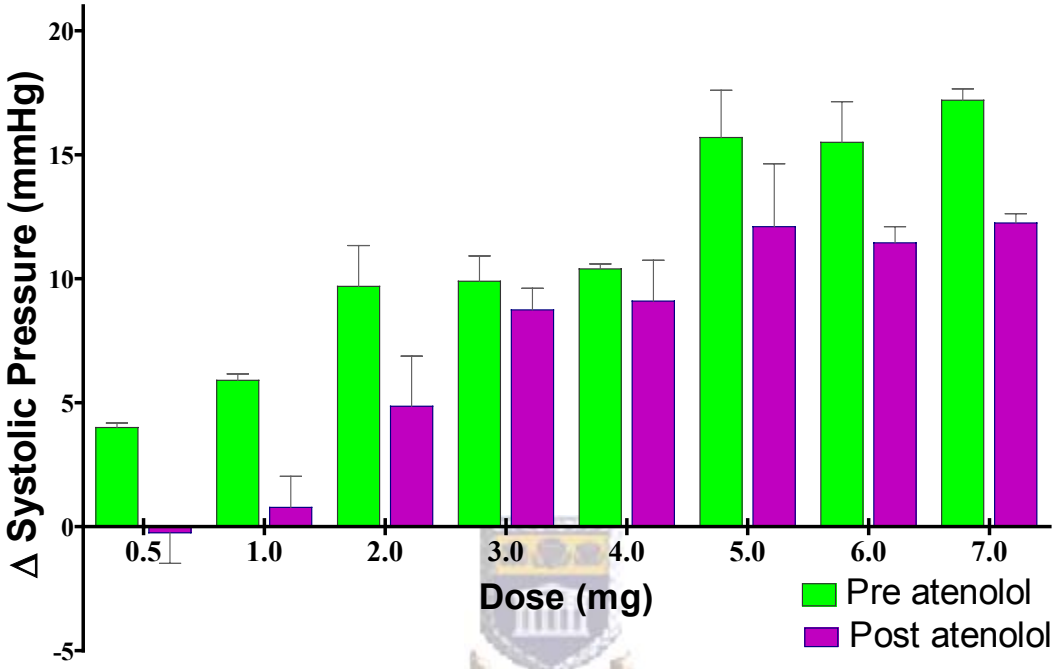

Figure 4.5: Effect of atenolol on the change in systolic pressure produced by the crude aqueous extract of *Leonotis leonurus* on systolic pressure.

Table 4.1: Means, standard deviations and p values of systolic pressure for crude aqueous extract of *Leonotis leonurus* pre and post administration of atenolol (2mg) (n=6).

Dose (mg)	Δ Systolic Pressure (mmHg) Pre atenolol administration Mean ± SD	Δ Systolic Pressure (mmHg) Post atenolol administration Mean ± SD	*P* value
0.5	4.00 ± 0.180	-0.25 ± 1.23	0.0115
1.0	5.90 ± 0.260	0.78 ± 1.25	<0.0001
2.0	9.70 ± 1.640	4.86 ± 2.02	0.3119
3.0	9.90 ± 1.020	8.75 ± 0.87	0.1113
4.0	11.40 ± 0.500	9.10 ± 1.65	0.1102
5.0	15.70 ± 2.900	12.10 ± 2.54	0.3110
6.0	13.50 ± 1.640	11.45 ± 0.65	0.0309
7.0	17.20 ± 0.460	12.25 ± 0.38	0.0151

Crude aqueous extract of *Leonotis leonurus* and prazosin

As shown in figure 4.6, administration of prazosin (60μg/kg) produced a decrease in the change in systolic pressure by *Leonotis leonurus* at all doses (0.5mg – 7.0mg). This decrease was dose dependent, with the change in systolic pressure decreased from

1.724mmHg ± 0.4800 to 0.54mmHg ± 0.3727 at the lowest dose (0.5mg) and from 18.88mmHg ± 2.53 to 8.922mmHg ± 1.075 at the highest dose (7.0mg) of *Leonotis leonurus*.

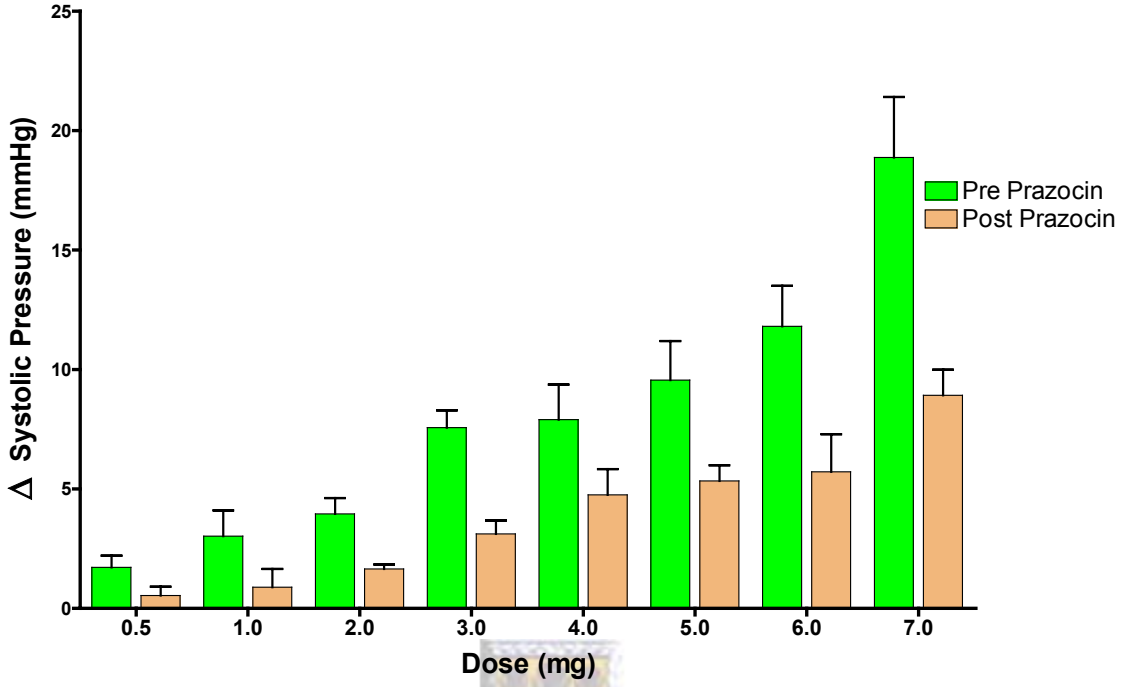

Figure 4.6: Effect of prazosin on the change in systolic pressure produced by the crude aqueous extract of *Leonotis leonurus* on systolic pressure.

Table 4.2: Means, standard deviations and *p* values of systolic pressure for crude aqueous extract of *Leonotis leonurus* pre and post administration of prazosin (60µg/ml) (n=6).

Dose (mg)	Δ Systolic Pressure (mmHg) pre prazosin administration Mean ± SD	Δ Systolic Pressure (mmHg) post prazosin administration Mean ± SD	*P* value
0.5	1.72 ± 0.48	0.54 ± 0.373	0.0104
1.0	3.02 ± 1.08	0.88 ± 0.765	0.0159
2.0	3.96 ± 0.66	1.65 ± 0.189	0.0082
3.0	7.57 ± 0.72	3.12 ± 0.562	0.0001
4.0	7.90 ± 1.47	4.76 ± 1.077	0.0791
5.0	9.56 ± 1.63	5.34 ± 0.654	0.0280
6.0	11.81 ± 1.69	5.72 ± 1.571	0.0171
7.0	18.88 ± 2.53	8.92 ± 1.075	0.0248

Fraction C

Fraction C produced a dose dependent increase in systolic pressure (fig. 4.7). The lowest dose, 0.01mg increased systolic pressure by 5.06mmHg ± 0.68, while the highest dose of 0.05mg increased systolic pressure by 24.2mmHg ± 1.865. The changes in systolic

pressure were statistically significant for all doses (p= 0.0003 for 0.01mg; p<0.0001 for 0.02mg – 0.05mg).

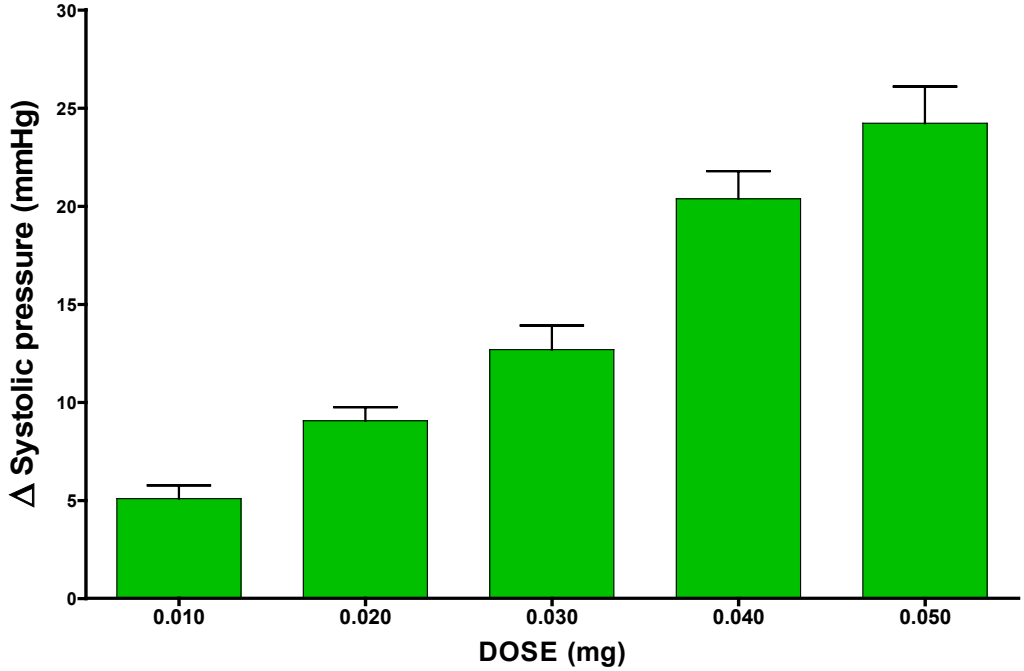

Figure 4.7: Effect of fraction C on systolic pressure.

4.2.1.2 Effect on Diastolic pressure

Adrenaline

Adrenaline administered in the dose range of 0.01mg – 0.08mg, had a dose dependent effect on the diastolic pressure, with the change in pressure increasing as the dose was increased (fig. 4.8). At low doses (0.01mg), there was a 2.1mmHg ± 0.51 increase in diastolic pressure, and at the highest dose administered (0.08mg), there was a 76.4mmHg ± 4.87 increase in diastolic pressure. These changes where statistically significant (p= 0.0252; 0.0077; 0.0012; 0.0030; 0.0042; 0.0039; <0.0001; 0.0006 for 0.01mg – 0.08mg respectively).

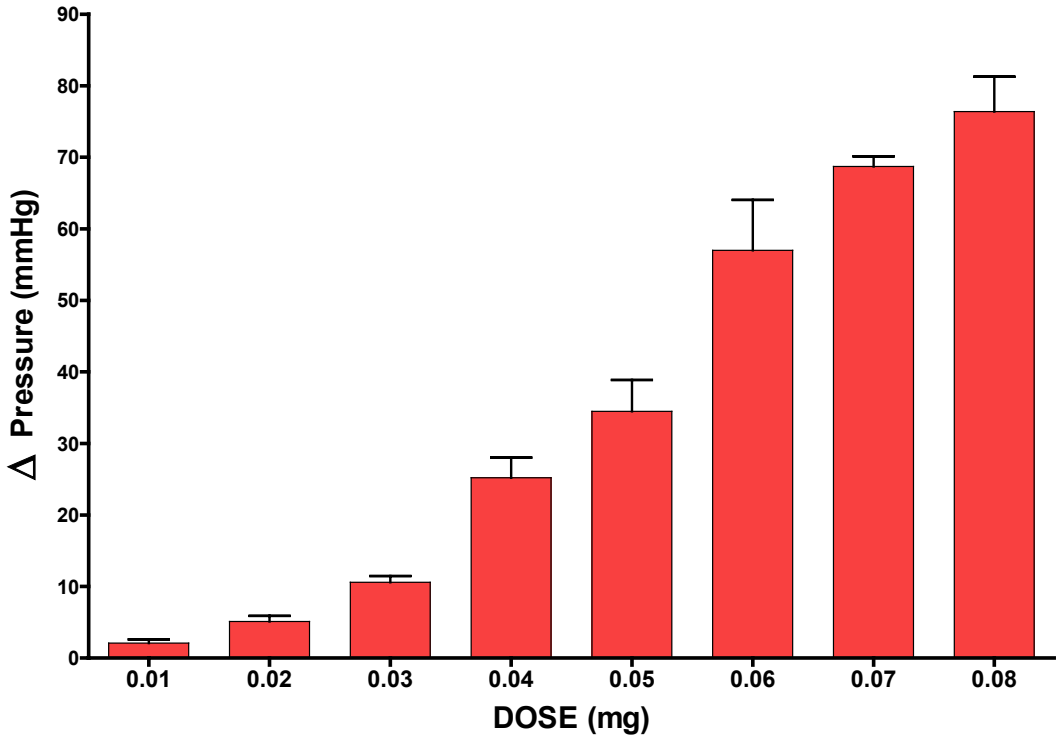

Figure 4.8: Effect of adrenaline on diastolic pressure.

Adrenaline had a similar effect on both systolic and diastolic pressures.

Atenolol

Administered at the dose range of 0.1mg – 3.0mg, atenolol produced a dose dependent decrease in diastolic pressure (fig. 4.9). The lowest dose (0.1mg) decreased diastolic pressure by 0.88mmHg ± 1.77, while the largest dose of 3.0mg decreased diastolic pressure by 13.01mmHg ± 4.02. The decrease in diastolic pressure was significant at higher doses (p= 0.0105; 0.0390; 0.0231 for 1.0, 2.0 and 3.0mg respectively) while at lower dose (0.1mg and 0.5mg) the change in pressure was not statistically significant (p= 0.6411 and 0.1544).

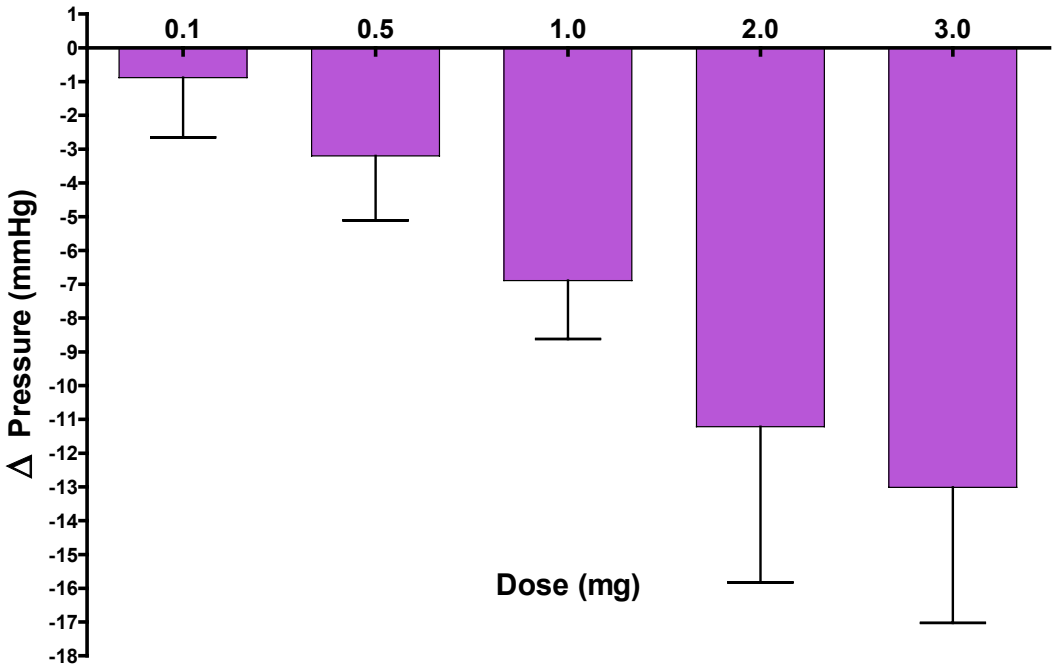

Figure 4.9: Effect of atenolol on diastolic pressure.

The effect of atenolol was similar on both systolic and diastolic pressures.

Prazosin

Prazosin produced a decrease in diastolic pressure that was dose dependent (20µg/kg – 100µg/kg). As shown in figure 4.10, a 20µg/kg dose decreased diastolic pressure by 5.64mmHg ± 2.22, while the highest dose (100µg/kg) decreased systolic pressure by 16.88mmHg±4.96.

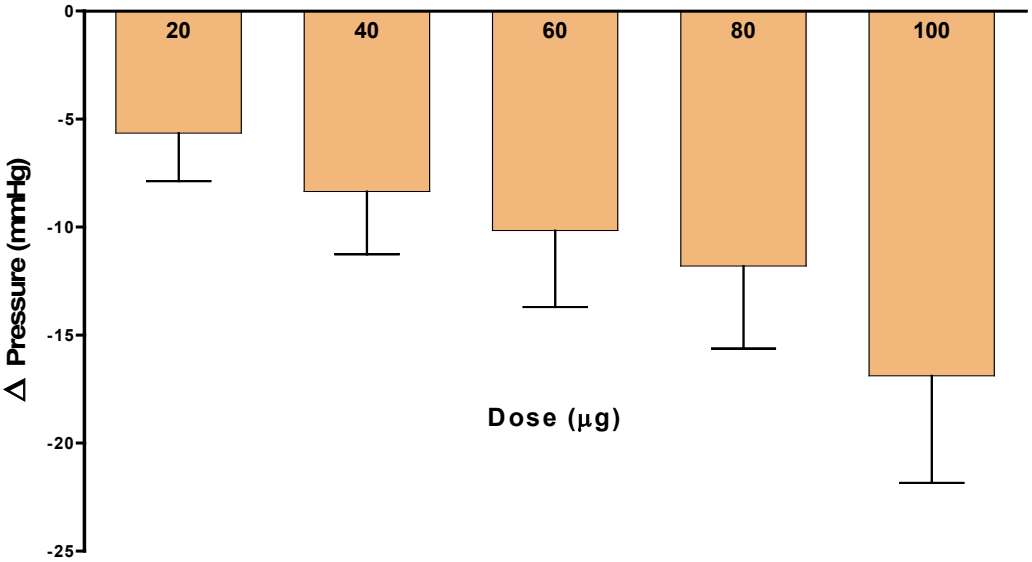

Figure 4.10: Effect of prazosin on diastolic pressure.

Just like adrenaline and atenolol, prazosin had a similar dose dependent effect on on diastolic pressure as it had on systolic pressure.

Crude aqueous extract of *Leonotis leonurus*

The crude aqueous extract of *L. leonurus* had a dose dependent effect on the diastolic pressure (fig. 4.11). At the doses (0.5mg and 1.0mg), there was a decrease in diastolic pressure (-1.7mmHg ± 0.29 and -1.2mmHg ± 1.18), while at higher doses (2.0mg - 7.0mg) there was a dose dependent increase in diastolic pressure (1.90mmHg ± 0.66 - 12.3mmHg ± 1.72). The decrease in diastolic pressure was significant at the lowest dose (0.5mg) (p=0.0098), but at the two doses bordering the change from decrease to increase in diastolic pressure (1.0mg and 2.0mg), change in diastolic pressure was not statistically significant (p=0.3942 and 0.7071 respectively). At higher doses (2.0mg – 7.0mg) change in diastolic pressure was statistically significant (p=0.0051; 0.2047; 0.2982; 0.0030; 0.0056 for 2.0mg, 3.0mg, 4.0mg, 50mg, 6.0mg and 7.0mg respectively).

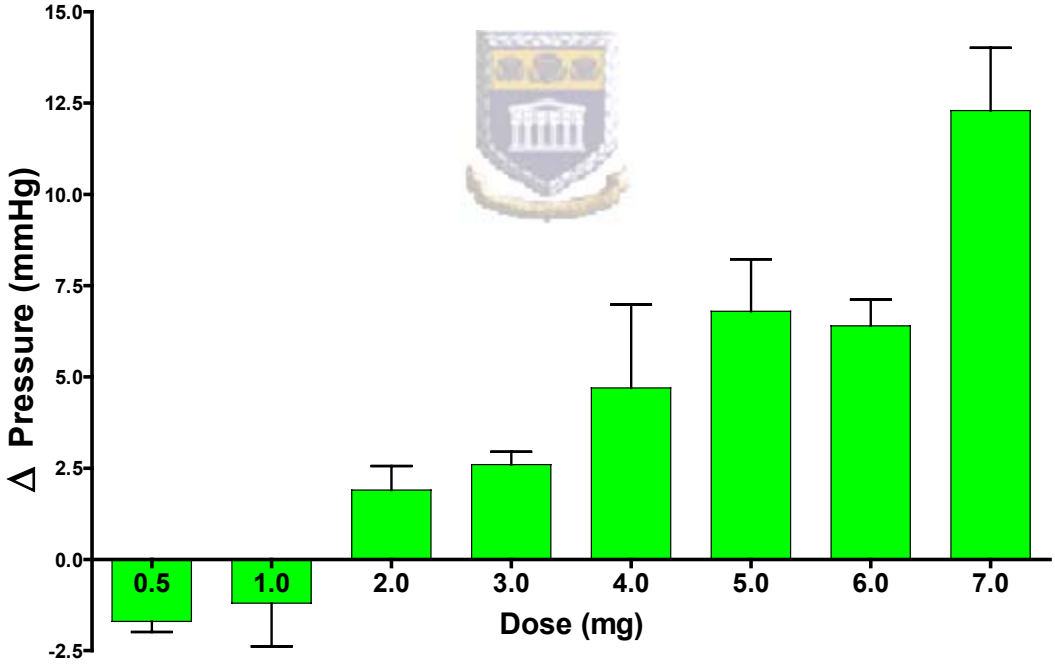

Figure 4.11: Effect of crude aqueous extract of *Leonotis leonurus* on diastolic pressure.

At the lowest doses, 0.5mg and 1.0mg, the effect of the crude aqueous extract on diastolic pressure was opposite the effect on systolic pressure. There was a decrease in diastolic pressure, while there was an increase in systolic pressure. From 2.0mg to 7.0mg, the effects on systolic and diastolic pressures were similar.

Crude aqueous extract of *Leonotis leonurus* and atenolol

Figure 4.12 below shows the changes in diastolic pressure caused by the crude aqueous extract of *Leonotis leonurus* before and after administration of atenolol (2mg). At the lower doses (0.5 and 1.0mg), atenolol caused a further decrease in the diastolic pressure though this was not statistically significant (from -1.7mmHg ± 0.29 to –2.2mmHg ± 0.4 for 0.5mg and from –1.2mmHg ± 1.18 to –2.3mmHg ± 1.09 for 1.0mg; p=0.2514 and 0.0876 respectively). With 2.0mg dose however, atenolol reversed the increase in pressure (1.9mmHg ± 1.66) to a decrease (-1.38mmHg ± 0.35). At higher doses (3.0mg – 7.0mg) there was an increase in pressure with the post atenolol administration of *L. leonurus*, however this increase was less than that achieved when *L. leonurus* was administered alone.

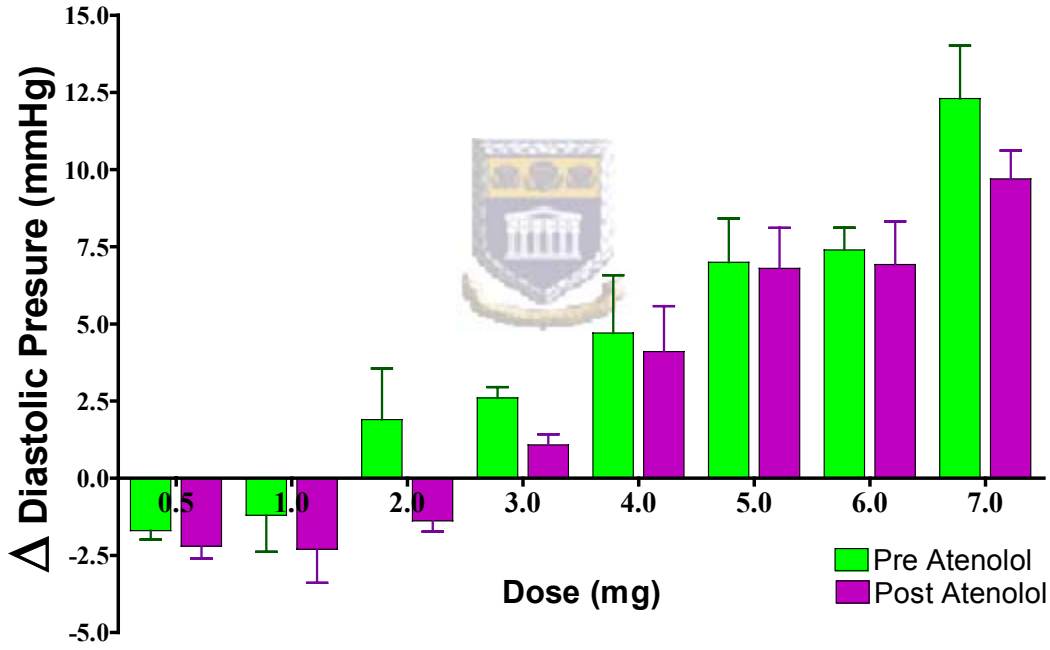

Figure 4.12: Effect of atenolol on the change in diastolic pressure produced by the crude aqueous extract of *Leonotis leonurus*.

Pre-administration of atenolol affected the systolic and diastolic response to the crude aqueous extract of Leonotis leonurus. At 0.5mg, 1.0mg and 2.0mg, there was a negative change in diastolic pressure, while there was a reduction in the increase in systolic pressure. From 3.0mg to 7.0mg, there was however a similar decrease in the increase in both systolic and diastolic pressures.

Table 4.3: Means, standard deviations and *p* values of diastolic pressure for crude aqueous extract of *Leonotis leonurus* pre and post administration of atenolol (2mg) (n=6).

Dose (mg)	Δ Diastolic Pressure (mmHg) Pre atenolol administration Mean ± SD	Δ Diastolic Pressure (mmHg) Post atenolol administration Mean ± SD	*P* value
0.5	-1.70 ± 0.29	-2.20 ± 0.40	0.2514
1.0	-1.20 ± 1.18	-2.30 ± 1.09	0.0876
2.0	1.90 ± 1.66	-1.38 ± 0.35	0.0805
3.0	2.60 ± 0.35	1.08 ± 0.34	0.0180
4.0	4.70 ± 1.88	4.10 ± 1.48	0.4112
5.0	7.00 ± 1.42	6.80 ± 1.31	0.9184
6.0	7.40 ± 0.72	6.92 ± 1.40	0.1879
7.0	12.30 ± 1.72	9.70 ± 0.92	0.0442

Crude aqueous extract of Leonotis leonurus and prazosin

Figure 4.13 below shows the changes in diastolic pressure caused by the crude aqueous extract of *L. leonurus* before and after administration of prazosin (60µg/kg). At the lowest dose (0.5mg), prazosin caused a further decrease in the diastolic pressure though not statistically significant (from -1.832mmHg ± 1.152 to –5.646mmHg ± 0.5573; *p*=0.0764). Between the 1.0mg and 2.0mg dose however, prazosin reversed the increase in pressure (0.964mmHg ± 0.9214 and 1.428mmHg ± 0.6942) to a decrease (-4.018mmHg ± 0.6553 and –1.082mmHg ± 0.4620). At higher doses (3.0mg – 7.0mg) diastolic pressure increase with the post prazosin administration of *L. leonurus*, however this increase was less than that achieved when *L. leonurus* was administered alone.

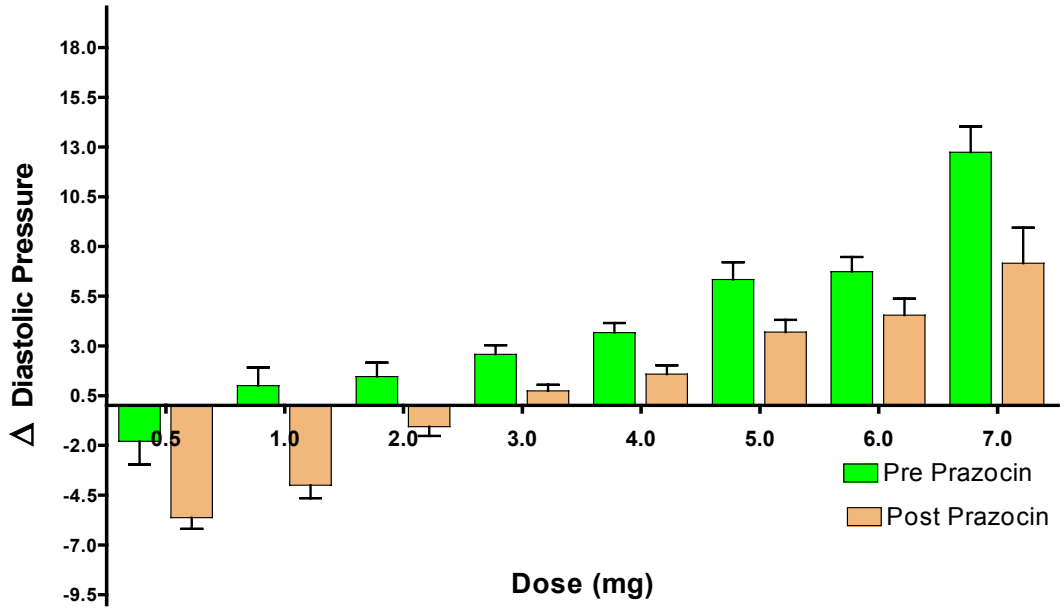

Figure 4.13: Effect of prazosin on the change in diastolic pressure produced by the crude aqueous extract of *Leonotis leonurus*.

Table 4.4: Means, standard deviations and *p* values of diastolic pressure for crude aqueous extract of *Leonotis leonurus* pre and post administration of prazosin (60µg/ml) (n=6).

Dose (mg)	Δ Diastolic Pressure (mmHg) Pre prazosin administration Mean ± SD	Δ Diastolic Pressure (mmHg) Post prazosin administration Mean ± SD	*P* value
0.5	-1.83 ± 1.152	-5.65 ± 0.557	0.0764
1.0	0.96 ± 0.921	-4.02 ± 0.655	0.0567
2.0	1.43 ± 0.695	-1.08 ± 0.462	0.0267
3.0	2.54 ± 0.459	0.72 ± 0.306	0.0097
4.0	3.64 ± 0.473	1.56 ± 0.442	0.0457
5.0	6.30 ± 0.867	3.68 ± 0.602	0.1173
6.0	6.69 ± 0.743	4.53 ± 0.825	0.1032
7.0	12.68 ± 1.295	7.14 ± 1.775	0.0155

Pre-administration of prazosin affected the systolic and diastolic response to the crude aqueous extract of Leonotis leonurus. At 0.5mg, 1.0mg and 2.0mg, there was a negative change in diastolic pressure, while there was a reduction in the increase in systolic pressure. From 3.0mg to 7.0mg, there was however a similar decrease in the increase in both systolic and diastolic pressures.

Fraction C

Fraction C of the methanol extract of *L. leonurus* had a dose dependent effect on the diastolic pressure (fig. 4.14). There was an increase in the change in diastolic pressure with an increase in dose. At the lowest dose (0.01mg) this change is diastolic pressure was not significant (0.0243mmHg ± 0.6733; p=0.2189), while at higher doses there was a significant change in dose (3.7390mmHg ± 1.157, 6.727mmHg ± 0.7731, 11.81mmHg ± 1.467 and 16.45mmHg ± 1.737: p=0.2189; 0.0179; 0.0001; 0.0002 and p<0.0001 for doses 0.02, 003, 0.04 and 0.05mg respectively).

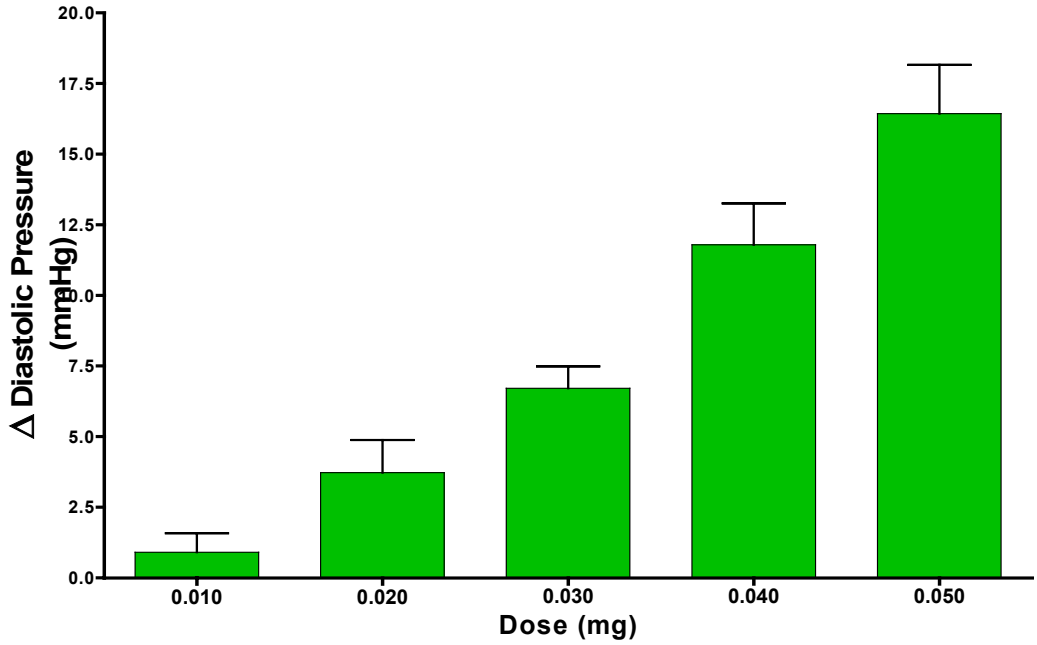

Figure 4.14: Effect of fraction C of the methanol extract of *Leonotis leonurus* on diastolic pressure.

The effect of fraction C on diastolic pressure was similar to its effect on systolic pressure.

4.1.1.3. Effect on Mean arterial pressure

Adrenaline

Within the dose range given (0.01mg – 0.08mg), adrenaline had a dose dependent effect on the mean arterial pressure, with the change in pressure increasing as the dose was increased (fig. 4.15). The smallest change (1.8mmHg ± 0.39) occurred at the lowest dose (0.01mg), while the largest change in mean arterial pressure (111.8mmHg ± 2.21) occurred at the highest dose administered (0.08mg). At all doses, the change in mean arterial pressure was statistically significant (p= 0.0210; 0.0002; 0.0014; 0.0007; 0.0007; 0.0002; p<0.0001and p<0.0001 for dose 0.01 to 0.08mg respectively).

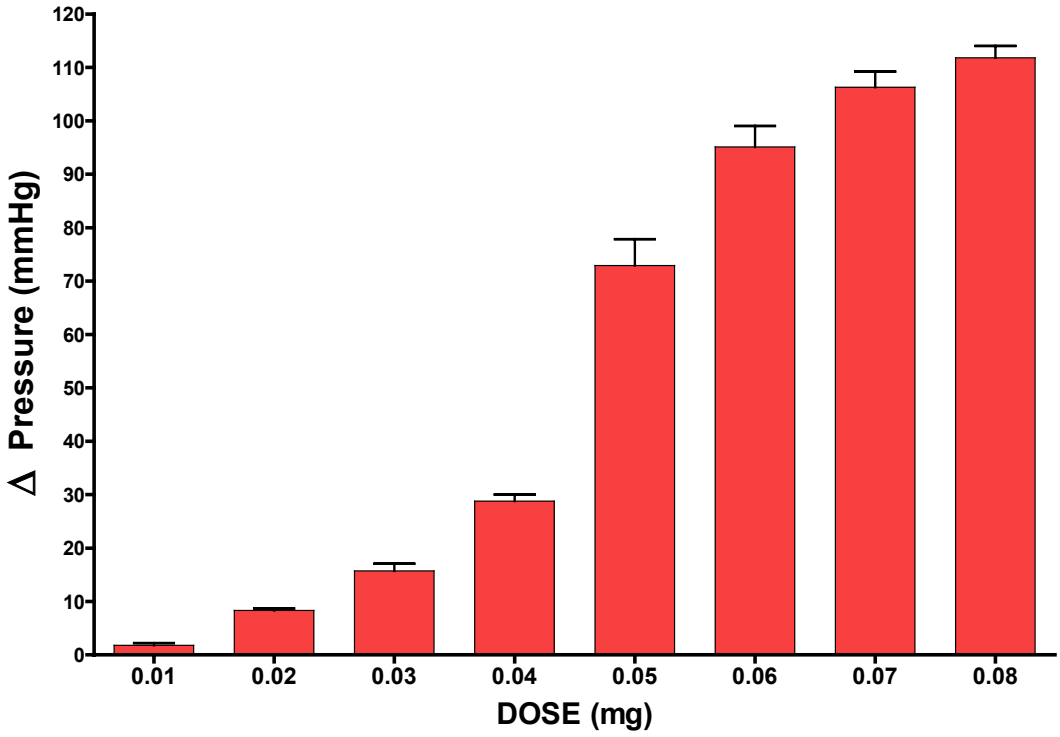

Figure 4.15: Effect of adrenaline on mean arterial pressure.

Atenolol

At all administered doses (0.1mg – 3.0mg), atenolol produced a decrease in mean arterial pressure that was dose dependent (fig. 4.16). At the lowest dose (0.1mg) mean arterial pressure was decreased by 1.41mmHg ± 1.56; while at the highest dose of 3.0mg, mean arterial pressure decreased by 13.83mmHg ± 3.79.

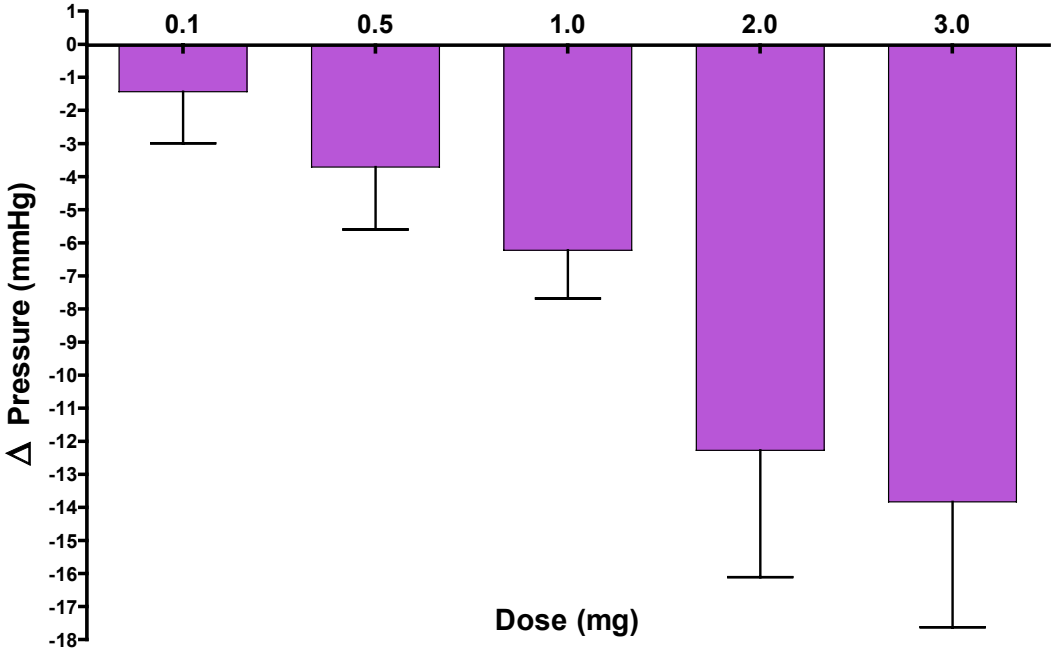

Figure 4.16: Effect of atenolol on mean arterial pressure.

Prazosin

Figure 4.17 shows the effect of prazosin (20µg/kg – 100µg/kg) on mean arterial pressure. Prazosin at all doses administered produced a dose dependent decrease in mean arterial pressure. The lowest dose (20µg/kg) decreased mean arterial pressure by 7.38mmHg ± 3.67, while the highest dose (100µg/kg) decreased mean arterial pressure by 20.94mmHg ± 5.94.

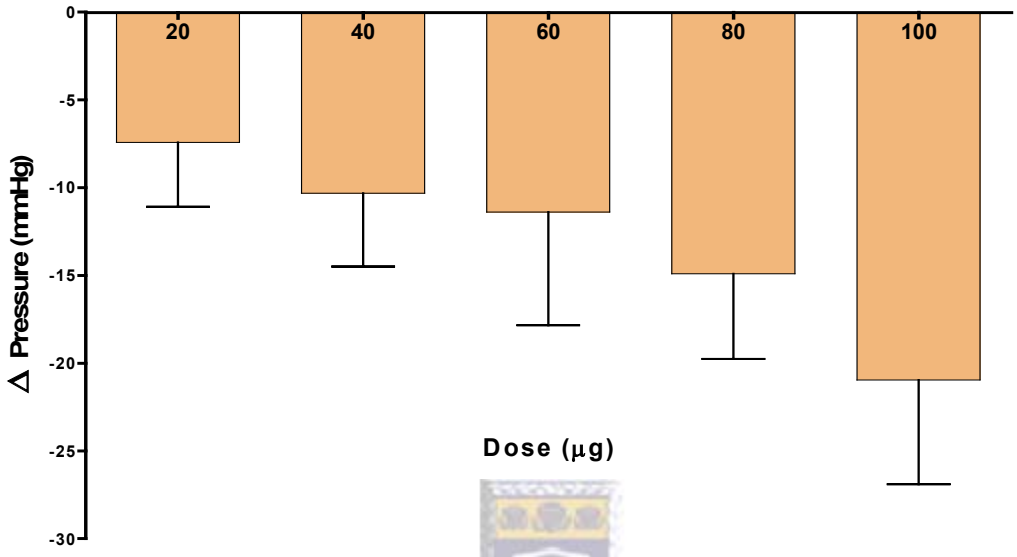

Figure 4.17: Effect of prazosin on mean arterial pressure.

Crude aqueous extract of *Leonotis leonurus*

The crude aqueous extract of *L. leonurus* had a dose dependent effect on mean arterial pressure (fig. 4.18). At all doses (0.5mg - 7.0mg), there was an increase in mean arterial pressure (2.10mmHg ± 0.220; 3.50mmHg ± 0.350; 7.10mmHg ± 2.210; 7.70mmHg ± 1.280; 9.10mmHg ± 1.260; 11.70mmHg ± 4.140; 11.20mmHg ± 1.320; 15.60mmHg ± 0.860). These changes were statistically significant (p=0.0024; 0.0021; 0.0482; 0.0091; 0.0055; 0.0661; 0.00350; and 0.0004for 0.5mg, 1.0mg, 2.0mg, 3.0mg, 4.0mg, 50mg, 6.0mg and 7.0mg respectively).

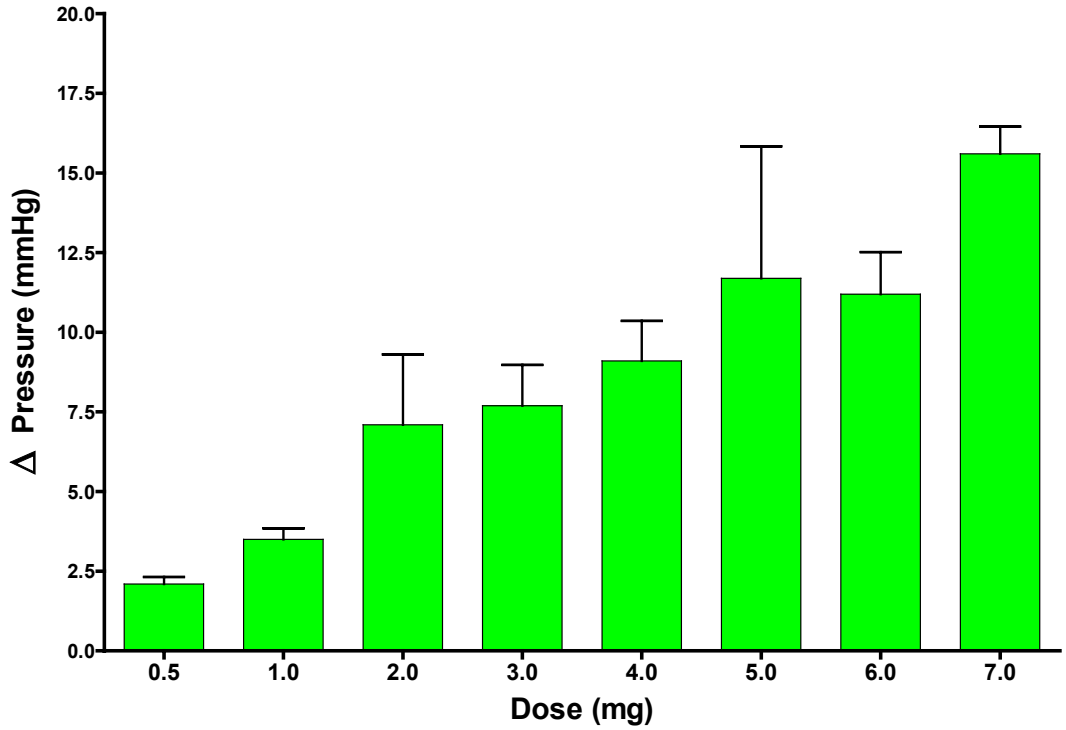

Figure 4.18: Effect crude aqueous extract of *Leonotis leonurus* on mean arterial pressure.

Crude aqueous extract of *Leonotis leonurus* and atenolol

Figure 4.19 below shows the changes in mean arterial pressure caused by the crude aqueous extract of *Leonotis leonurus* (0.5mg – 7.0mg) before and after administration of atenolol (2mg). At the lower doses (0.5 and 1.0mg), atenolol administration reversed the increase in mean arterial pressure (2.1mmHg ± 0.22 and 3.5mmHg ± 0.35) to a significant decrease (0.90mmHg ± 0.71 and 0.28mmHg ± 1.29; p=0.0077 and 0.0031 respectively). Between 2.0mg and 5.0mg, atenolol administration reduced the increase in mean arterial pressure produced by the crude aqueous extract of Leonotis leonurus (from 7.1mmHg ± 2.21 to 3.88mmHg ± 2.16 for 2.0mg, from 7.7mmHg ± 1.28 to 6.15mmHg ± 0.07 for 3.0mg, from 9.1mmHg ± 1.26 to 7.45mmHg ± 1.31 for 4.0mg and from 11.7mmHg ± 1.14 to 10.4mmHg ± 1.63 for 5.0mg), though this was not statistically significant. With 6.0mg and 7.0mg doses however, the reduction in change in mean arterial pressure produced by pre administration of atenolol was statistically significant (11.2mmHg ± 1.32 to 7.93mmHg ± 0.92 and 15.6mmHg ± 0.86 to 9.9mmHg ± 0.38; p= 0.0217 and 0.0146 for the 6.0mg an 7.0mg dose respectively).

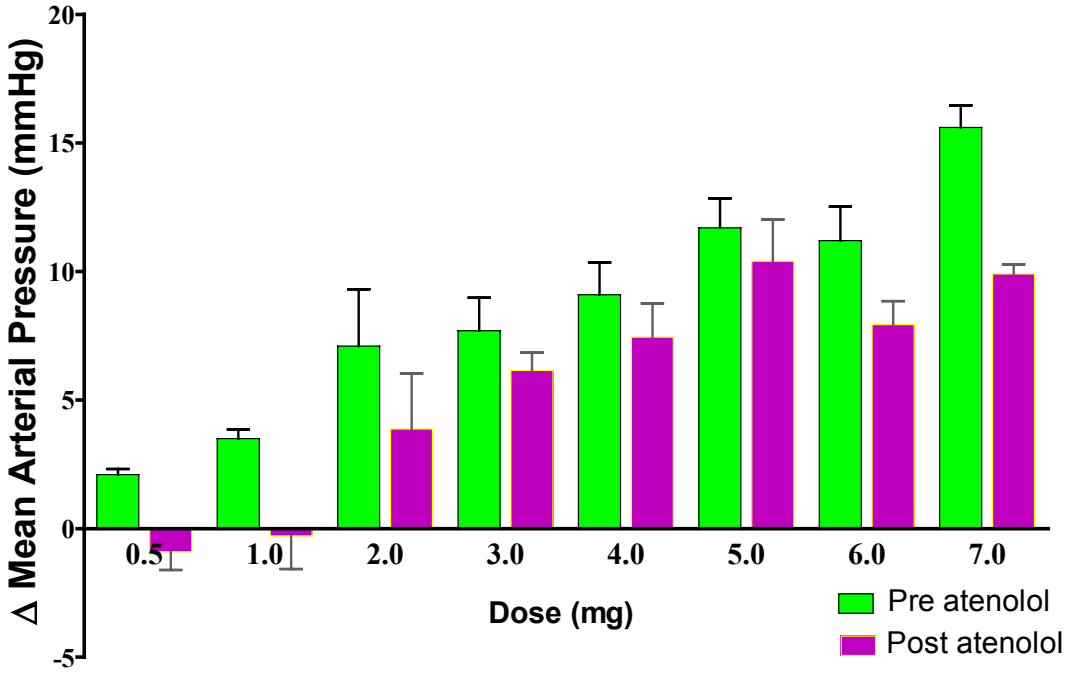

Figure 4.19: Effect of atenolol on the change in mean arterial pressure produced by the crude aqueous extract of *Leonotis leonurus*.

Table 4.4: Means, standard deviations and *p* values of mean arterial pressure for crude aqueous extract of *Leonotis leonurus* (0.5mg – 7.0mg) pre and post administration atenolol (2mg) (n=6).

Dose mg)	Δ MAP (mmHg) Pre atenolol administration Mean ± SD	Δ MAP (mmHg) Post atenolol administration Mean ± SD	*P* value
0.5	2.10 ± 0.22	-0.90 ± 0.71	0.0077
1.0	3.50 ± 0.35	-0.28 ± 1.29	0.0031
2.0	7.10 ± 2.21	3.88 ± 2.16	0.1473
3.0	7.70 ± 1.28	6.15 ± 0.70	0.1123
4.0	9.10 ± 1.26	7.45 ± 1.31	0.1077
5.0	11.70 ± 1.14	10.40 ± 1.63	0.3504
6.0	11.20 ± 1.32	7.93 ± 0.92	0.0217
7.0	15.60 ± 0.86	9.90 ± 0.38	0.0146

Crude aqueous extract of *Leonotis leonurus* and prazosin

Figure 4.20 below shows the changes in diastolic pressure caused by the crude aqueous extract of *Leonotis leonurus* before and after administration of prazosin (60µg/kg). At the low doses (0.5mg and 1.0mg), pre administration of prazosin (60µg/kg) reversed the increase in mean arterial pressure, produced by the crude aqueous extract of *Leonotis leonurus* (0.54mmHg ± 0.5027 to -1.52mmHg ± 0.3324 for 0.5mg *L. leonurus*, and 1.70mmHg ± 0.8919 to -0.75mmHg ± 0.6129 for 1.0mg *Leonotis leonurus*). At other doses (2.0mg – 7.0mg) there was an increase in pressure with the post prazosin

administration of *L. leonurus*, however this increase was less than that achieved when *L. leonurus* was administered alone.

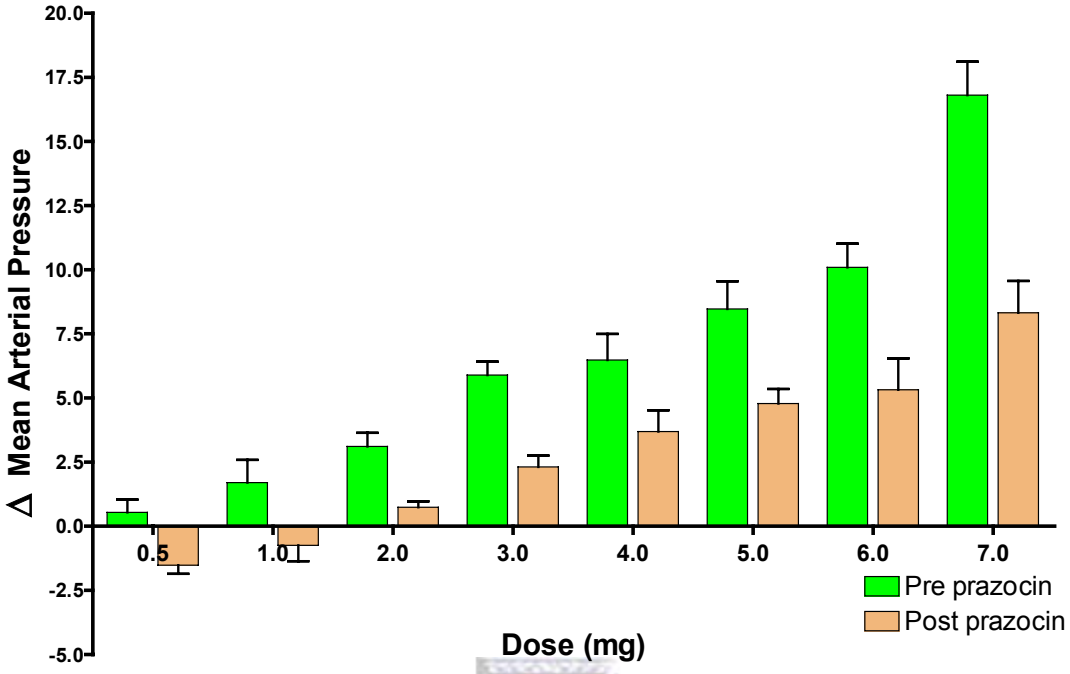

Figure 4.20: Effect of prazosin on the change in mean arterial pressure produced by the crude aqueous extract of *Leonotis leonurus*.

Table 4.5: Means, standard deviations and *p* values of Mean arterial pressure for crude aqueous extract of *Leonotis leonurus* (0.5mg – 7.0mg) pre and post administration prazosin (60µg/ml) (n=6).

Dose mg)	Δ MAP (mmHg) Pre prazosin administration Mean ± SD	Δ MAP (mmHg) Post prazosin administration Mean ± SD	*P* value
0.5	0.54 ± 0.5027	-1.52 ± 0.3324	0.0200
1.0	1.70 ± 0.8919	-0.75 ± 0.6129	0.0192
2.0	3.12 ± 0.5278	0.74 ± 0.2352	0.0032
3.0	5.89 ± 0.5270	2.32 ± 0.4388	0.0002
4.0	6.48 ± 1.0160	3.69 ± 0.8313	0.0583
5.0	8.47 ± 1.0730	4.79 ± 0.5681	0.0249
6.0	10.10 ± 0.9225	5.32 ± 1.2240	0.0182
7.0	16.81 ± 1.3000	8.33 ± 1.2410	0.0149

Fraction C

Figure 4.21 shows the dose dependent effect of fraction C of the methanol extract of *Leonotis leonurus* (0.01mg – 0.05mg) had on mean arterial pressure. There was an increase in the change in mean arterial pressure with an increase in dose. At all doses, change in mean arterial pressure was statistically significant (3.68mmHg ± 2.1870; 7.21mmHg ± 0.8639; 10.08mmHg ± 1.3140; 12.61mmHg ± 1.0060 and 19.27mmHg ±

1.0170: p= 0.0012; p<0.0001; p<0.0001; p<0.0001 and p<0.0001 for doses 0.01, 0.02, 003, 0.04 and 0.05mg respectively).

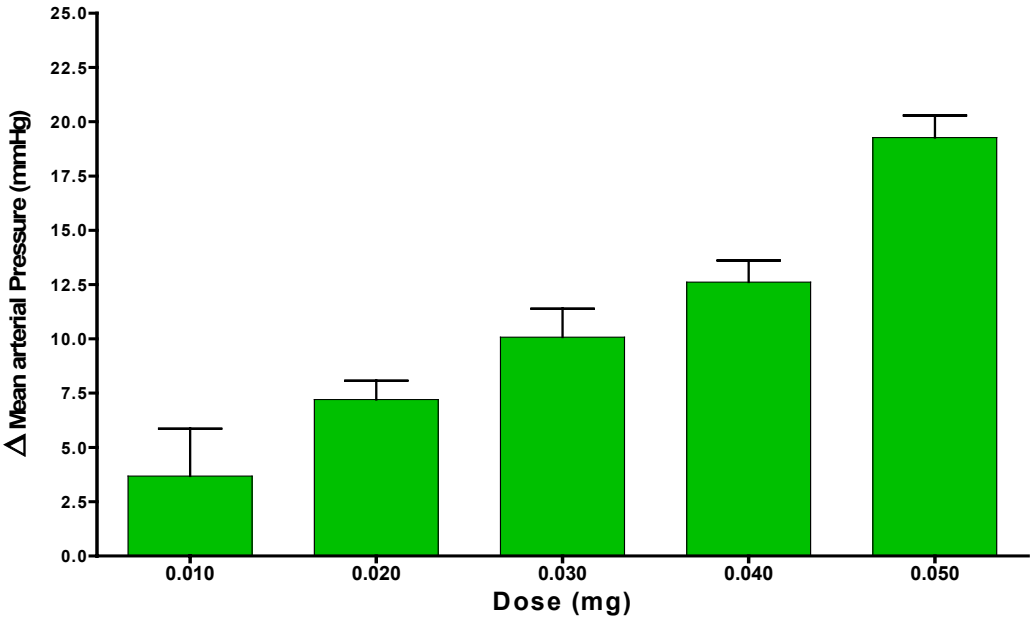

Figure 4.21: Effect of fraction C of the methanol extract of *Leonotis leonurus* on mean arterial pressure.

4.1.1.4 Effect on Heart rate

Adrenaline

Adrenaline (0.01mg – 0.08mg) produced a dose dependent increase in the heart rate with all the doses administered (fig. 4.22). The smallest change (8.5bpm ± 1.71) occurred at the lowest dose (0.01mg), while the largest change in heart rate (97.8bpm ± 6.69) occurred at the highest dose administered (0.08mg). At all doses, the change in mean arterial pressure was statistically significant (p= 0.0156; 0.0012; 0.0017; 0.0010; 0.0011; 0.0002; 0.0020; 0.0007 for adrenaline dose 0.01 to 0.08mg respectively).

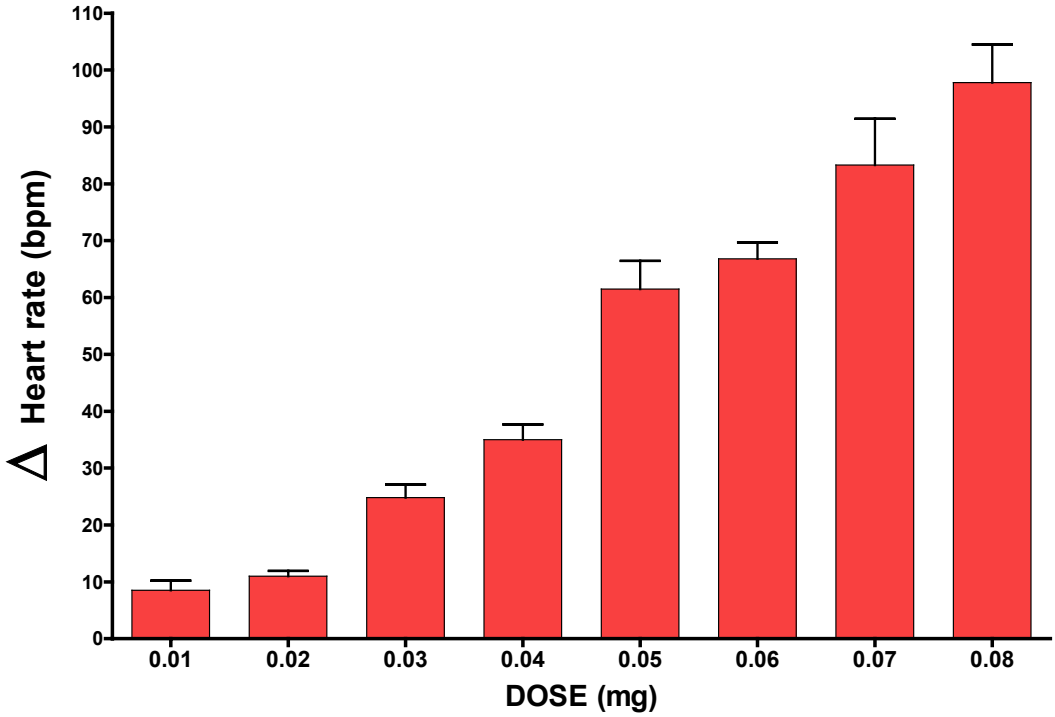

Figure 4.22: Effect of adrenaline on heart rate.

Adrenaline acts on both α_1 and β_1 receptors. Its action on these receptors produces the positive chronotropic effect seen in the figure above, as well as a positive inotropic effect.

Atenolol

As shown in figure 4.23 below, atenolol (0.1mg – 3.0mg) produced a dose dependent decrease in heart rate. At the lowest dose (0.1mg) heart rate was decreased by 9.55bpm ± 1.92; while at the highest dose of 3.0mg, heart rate decreased by 67.32bpm ± 8.08.

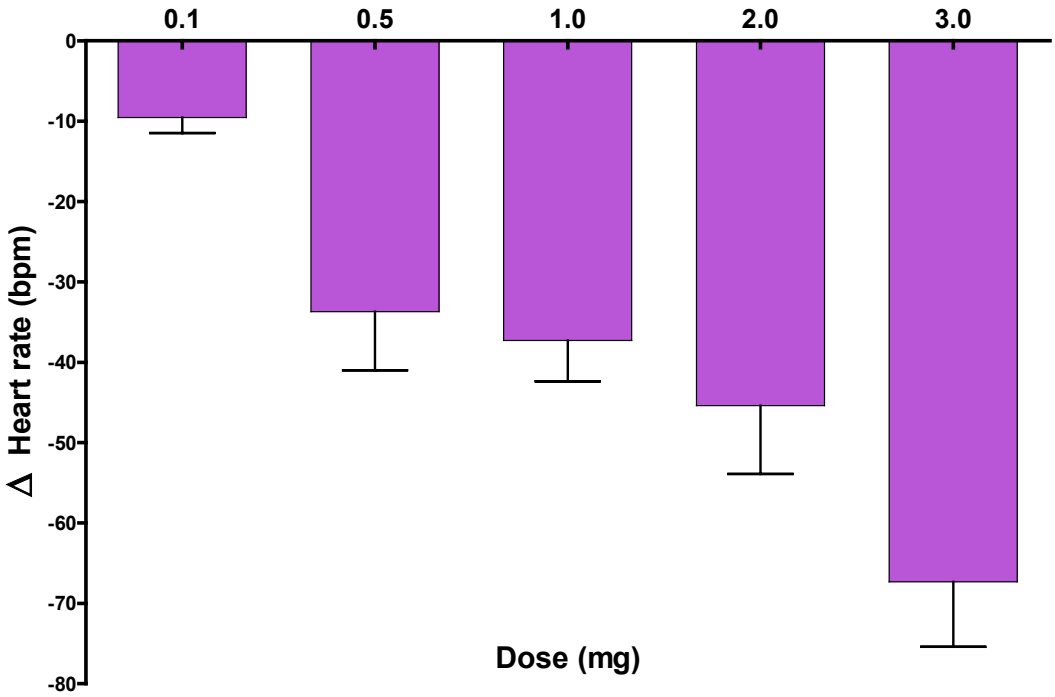

Figure 4.23: Effect of atenolol on heart rate.

Atenolol acting as a selective β_1 receptor antagonist produces a negative chronotropic and negative inotropic effect as seen in the figure above.

Prazosin

As shown in figure 4.24, prazosin (20µg/kg – 100µg/kg) produced a dose dependent increase in heart rate. The lowest dose (20µg/kg) increased heart rate by 1.32bpm ± 1.88, while the highest dose (100µg/kg) increased heart rate by 35.49bpm ± 4.21. At the lower doses the change in heart rate was not significant (p=0.8989 and p=0.5743 for 20µg/kg and 40µg/kg respectively), while at the higher doses, this change was significant (p= 0.0045; 0.0009; and 0.0004 for 60, 80 and 100µg/kg prazosin respectively).

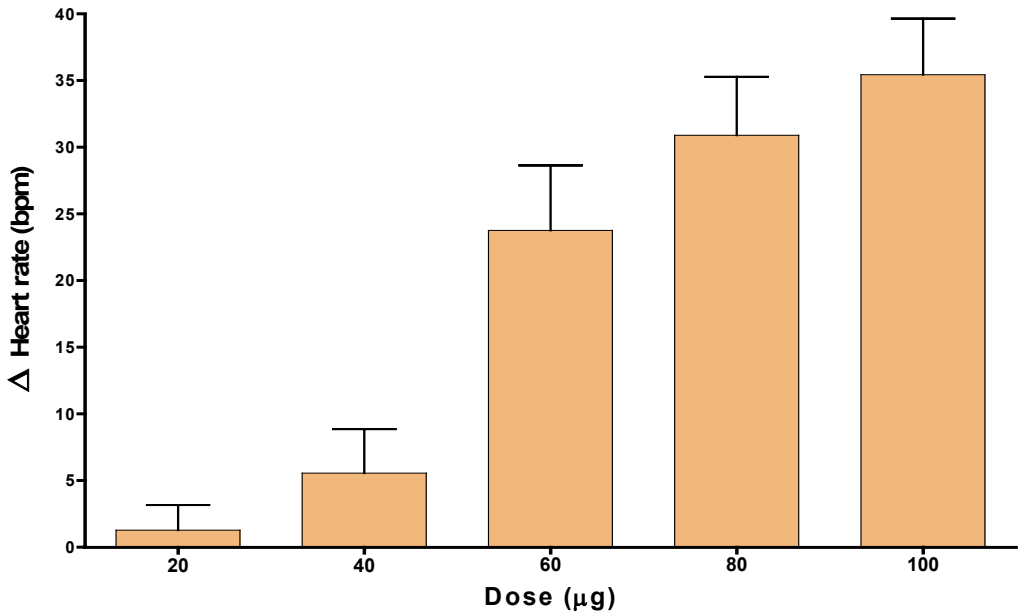

Figure 4.24: Effect of prazosin on heart rate.

Prazocin dilates the arteries via α_1 blockade, but at the same time, the drug also triggers a reflex tachycardia via the barorecptor reflex mechanism. The increase in heart rate observed with an increase in drug dose was probably due to an increased vasodilatory effect of the drug and an attendant increase in reflex tachycardia.

Crude aqueous extract of *Leonotis leonurus*

The crude aqueous extract of *Leonotis leonurus* had a dose dependent effect on the heart rate (fig 4.25). At low doses (0.5mg and 1.0mg), there was a dose dependent increase in heart rate (23.50bpm ± 10.17 and 7.00bpm ± 2.94), though not statistically significant (p=0.1039 and p=0.0978). At higher doses (2.0mg - 7.0mg) however, there was a decrease in heart rate (-1.00bpm ± 1.96 for 2mg; - 3.30bpm ± 1.04 for 3mg; -8.30bpm ± 0.45 for 4mg; -8.00bpm ± 2.10 for 5mg; -16.00bpm ± 0.82 for 6mg and –16.50bpm ± 0.68 for 7.0mg *Leonotis leonurus*: p=0.6447; 0.0021; p<0.0001; p=0.0285; 0.0003; and 0.0002 for 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0mg *Leonotis leonurus* respectively).

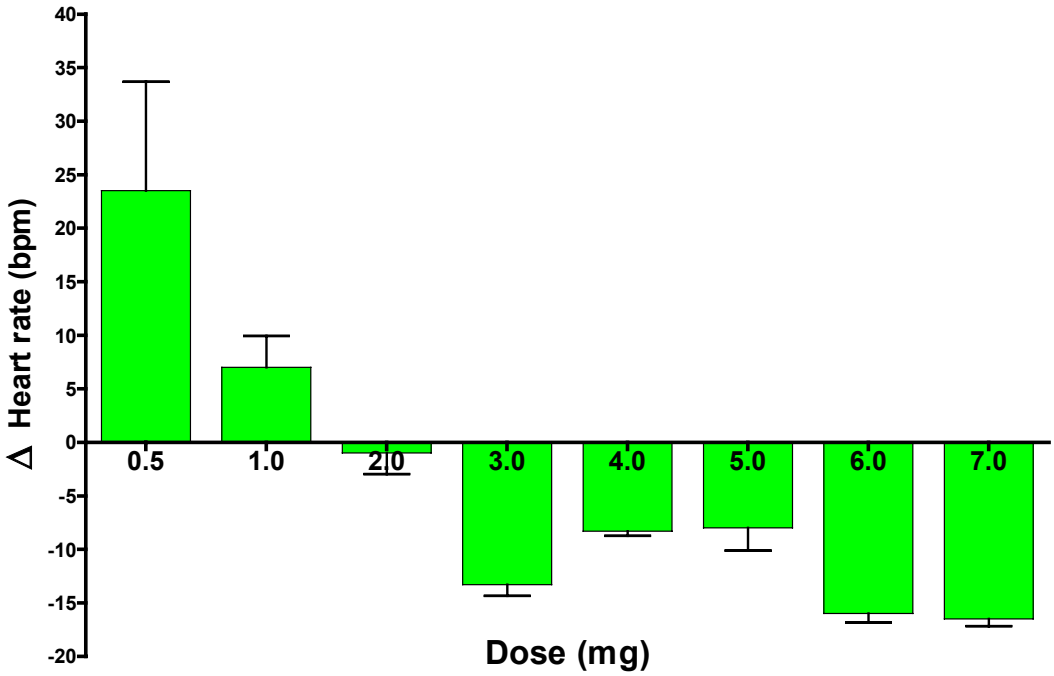

Figure 4.25: Effect of the crude aqueous extract of *Leonotis leonurus* on heart rate.

The low doses had a positive chronotropic effect on the heart, while higher doses had a negative chronotropic effect on the heart. This suggests that the extract either had a dose dependent effect on heart rate or that different constituent compounds exerted the net effect on heart rate at low and high doses.

Crude aqueous extract of *Leonotis leonurus* and atenolol

Figure 4.26 below shows the changes in heart rate caused by the crude aqueous extract of *Leonotis leonurus* (0.5mg – 7.0mg) before and after administration of atenolol (2mg). At low doses of *Leonotis* leonurus (0.5 and 1.0mg), atenolol reversed the increase (23.5bpm ± 10.17 and 7.0bpm ± 2.94 for 0.5mg and 1.0mg respectively) produced by the extract, to a decrease (-10.50bpm ± 2.02 and -10.25bpm ± 2.02 for 0.5mg and 1.0mg respectively) in heart rate, which was statistically significant ($p=0.0007$ and $p=0.0013$ for 0.5mg and 1.0mg respectively). When the dose was increased (2.0mg and above), pre administration of atenolol (2mg) increased the negative change in heart rate produced by the extract alone.

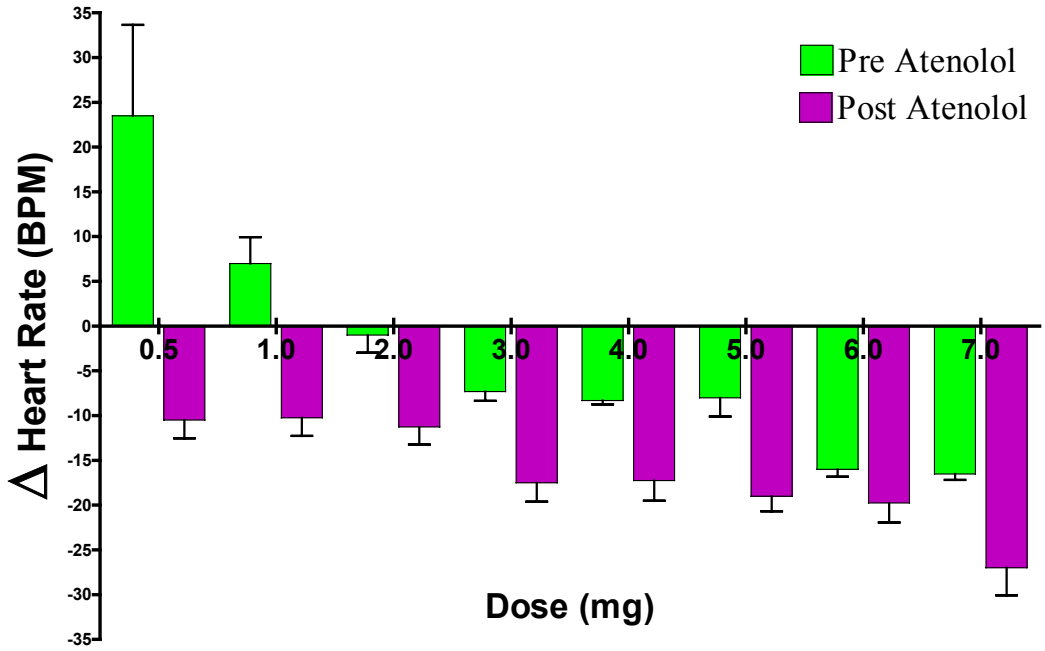

Figure 4.26: Effect of atenolol on the change in heart rate produced by the crude aqueous extract of *Leonotis leonurus*.

Table 4.6: Means, standard deviations and *p* values of heart rate for crude aqueous extract of *Leonotis leonurus* (0.5mg –7.0mg) pre and post administration atenolol (2mg) (n=6).

Dose (mg)	Δ Heart Rate (b.p.m) Pre atenolol administration Mean ± SD	Δ Heart Rate (b.p.m) Post atenolol administration Mean ± SD	*P* value
0.5	23.50 ± 10.17	-10.50 ± 2.02	0.0007
1.0	7.00 ± 2.94	-10.25 ± 2.02	0.0013
2.0	-1.00 ± 1.96	-11.25 ± 1.97	0.0024
3.0	-7.30 ± 1.04	-17.50 ± 2.10	0.0084
4.0	-8.30 ± 0.45	-17.25 ± 2.25	0.3181
5.0	-8.00 ± 2.10	-19.00 ± 1.68	0.0167
6.0	-16.00 ± 0.82	-19.75 ± 2.18	0.3332
7.0	-16.50 ± 0.68	-27.00 ± 3.08	0.2909

At the low doses (0.5 and 1.0mg) of the extract, atenolol blocked the positive chronotropic effect of the extract, producing a negative chronotropic effect. At higher doses, it also potentiated the negative chronotropic effect of the extract, suggesting that the extract affected the heart rate via β_1 receptors.

Crude aqueous extract of *Leonotis leonurus* and prazosin

Figure 4.27 below shows the changes in heart rate caused by the crude aqueous extract of *Leonotis leonurus* (0.5mg – 7.0mg) before and after administration of prazosin (60μg/kg). At the lowest doses (0.5mg and 1.0mg), pre administration of prazosin caused a reduction in the increase in heart rate produced by the crude extract of the plant

(19.86bpm ± 1.805 to –15.140bpm ± 0.9507 for 0.5mg, and from 8.726bpm ± 0.7714 to 6.856bpm ± 0.5820 for 1.0mg *Leonotis leonurus*). Pre administration of atenolol however reversed the increase in heart rate (1.178bpm ± 1.90) at the dose of 2.0mg Leonotis leonurus to a decrease of –8.726bpm ± 1.19. At higher doses, pre administration of atenolol caused a decrease in heart rate greater than that produced by the administration of the extract alone.

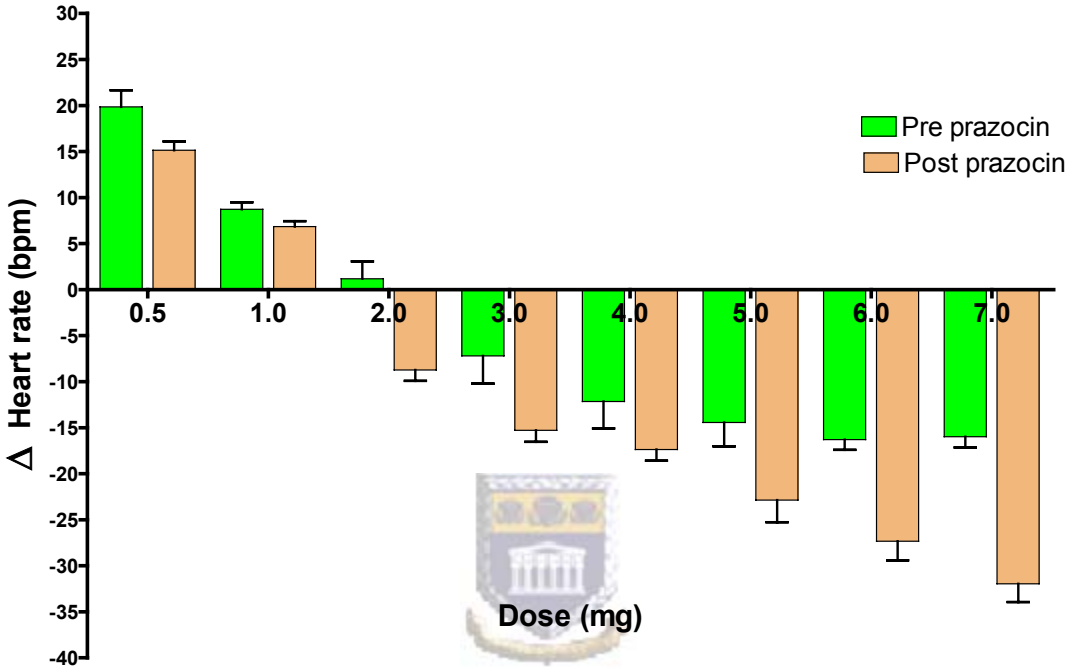

Figure 4.27: Effect of prazosin on the change in heart rate produced by the crude aqueous extract of *Leonotis leonurus*.

Table 4.7: Means, standard deviations and *p* values of heart rate for crude aqueous extract of *Leonotis leonurus* (0.5mg – 7.0mg) before and after administration of prazosin (60µg/kg) (n=6).

Dose (mg)	Δ Heart Rate (b.p.m) Pre prazosin administration Mean ± SD	Δ Heart Rate (b.p.m) Post prazosin administration Mean ± SD	*P* value
0.5	19.86 ± 1.8050	15.14 ± 0.9507	0.0257
1.0	8.73 ± 0.7714	8.86 ± 0.5820	0.9236
2.0	1.18 ± 1.9000	-8.73 ± 1.1900	0.0269
3.0	-7.19 ± 3.0350	-15.30 ± 1.2360	0.0459
4.0	-12.16 ± 2.9240	-17.38 ± 1.2120	0.1880
5.0	-14.43 ± 2.5870	-22.85 ± 2.4110	0.0841
6.0	-16.29 ± 1.1180	-27.34 ± 2.0620	0.0073
7.0	-15.99 ± 1.1670	-31.96 ± 1.9920	0.0005

At lower doses (0.5 and 1.0mg) of the crude extract, prazocin potentiated the increase in heart rate, possibly by an additive effect of the action of the crude extract and the reflex tachycardia produced by prazocin. At higher doses however, there was a potentiation of the decrease in heart rate when the extract was administered after prazocin.

Fraction C

Figure 4.28 below shows the effect of fraction C of the methanol extract of *Leonotis leonurus* (0.01mg – 0.05mg) on heart rate. The fraction had a dose dependent effect on heart rate. At the lowest dose (0.01mg), the fraction had a positive chronotropic effect on heart rate, increasing it by 3.68bpm ± 0.6429, though this change was not statistically significant (p= 0.1453). At higher doses (0.02 – 0.05mg), there was a significant increase in heart rate with the increase in dose (7.26bpm ± 0.7787, 10.68bpm ± 0.7885, 17.50bpm ± 1.2810 and 21.61bpm ± 1.4530: p=0.0002; 0.0003; p<0.0001; and p<0.0001 for doses 0.02, 003, 0.04 and 0.05mg respectively).

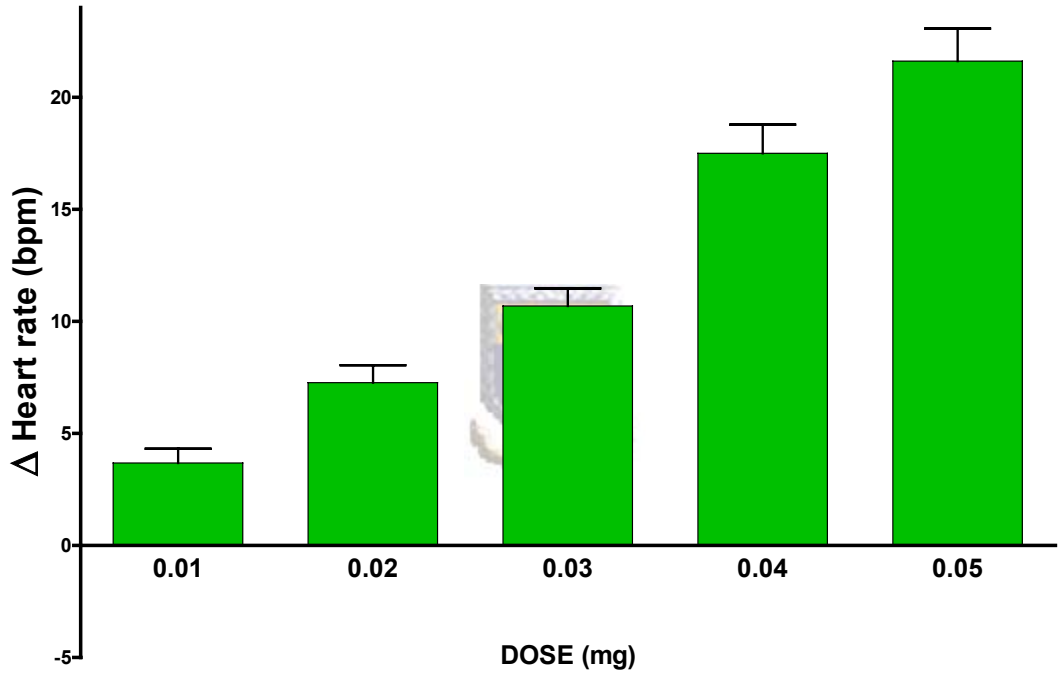

Figure 4.28: Effect of fraction C of the methanol extract of *Leonotis leonurus* on heart rate.

The effect of fraction C was quite the opposite of that observed with high doses of the crude aqueous extract. It increased heart rate at all doses, an effect opposite to the decrease in heart rate observed with the higher doses of the crude aqueous extract, however low doses of the crude extract had a similar effect on heart rate as the fraction. This suggests that different compounds bring about these effects.

4.2 *IN VITRO* EXPERIMENT RESULTS

This system is a modification of the working heart model that allows for the perfusion of the heart using two different perfusion mediums. The results in the following sections first show the validation of the two sides of the perfusion system and then the effect of Adrenaline (0.0001 – 0.0008mg/ml), 0.01mg/ml fraction C, combination of 0.01mg/ml fraction C and 0.07mg/ml atenolol on the systolic pressure (SP), diastolic pressure (DP), developed pressure (dp), coronary flow (Qe), aortic output (Qa) and cardiac output (CO). The concentration of 0.01mg/ml for fraction C was used because at higher concentrations, the fraction formed an unstable suspension, and precipitated upon standing. Lower doses could have been used but time constrains meant the use of one single dose of the fraction.

4.2.2 SYSTEM VALIDATION RESULTS

The isolated heart was perfused in the langendorff and work heart mode with Krebs-Hanseleit buffer solution from both sides of the double-sided working heart model to make sure that both sides gave similar readings for systolic pressure (SP), diastolic pressure (DP), developed pressure (Du), coronary flow (Qe), aortic output (Qa), and cardiac output (CO). Perfusion was randomized to eliminate the effect of time on the results. A sample size of 9 rat hearts was used.

4.2.1.1. Force of contraction

From figure 4.29, no significant difference in systolic pressure (p=0.6759), diastolic pressure (p=0.6131) and developed pressure (p=0.6292) indicating that there was no difference in function between both sides of the double-sided working heart system.

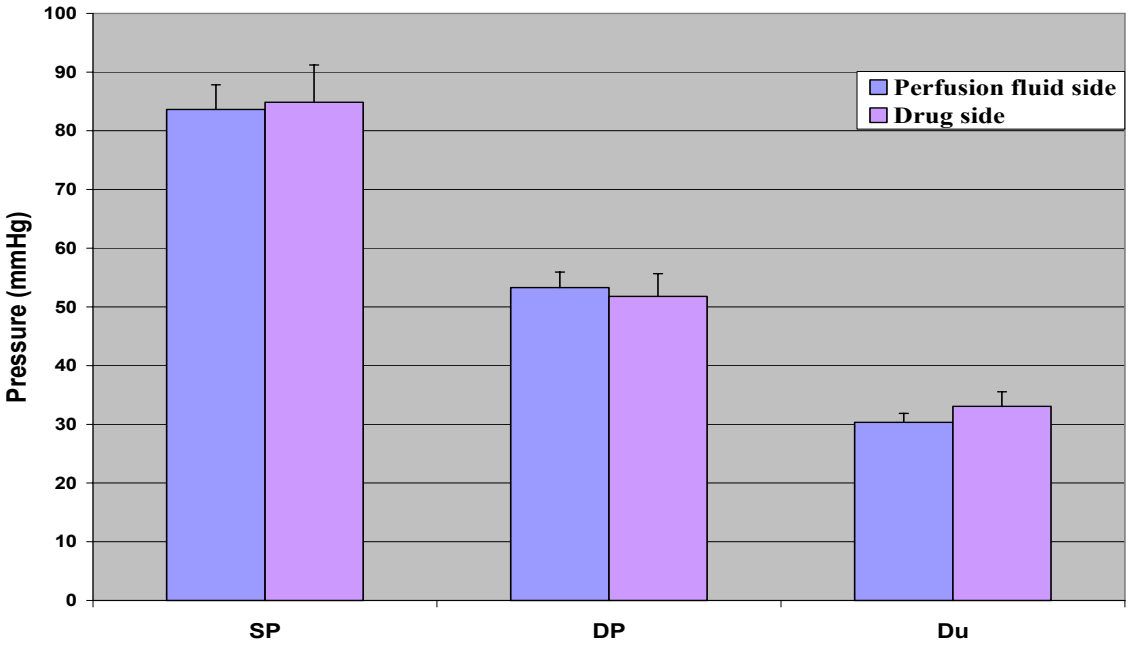

Figure 4.29: Systolic, diastolic and developed pressure readings for perfusion fluid and drug side of the double sided working heart system.

4.2.1.2. Coronary flow (Qe), Aortic output (Qa), and Cardiac output (CO)

Values for Qe, Qa and CO were measured after 10 minutes of perfusion, and the results (fig. 4.30) showed that there was no significant change in any of these parameters between the two sides of the system (*p* values – 0.8586, 0.5756 and 0.7672 for Qe, Qa and CO respectively).

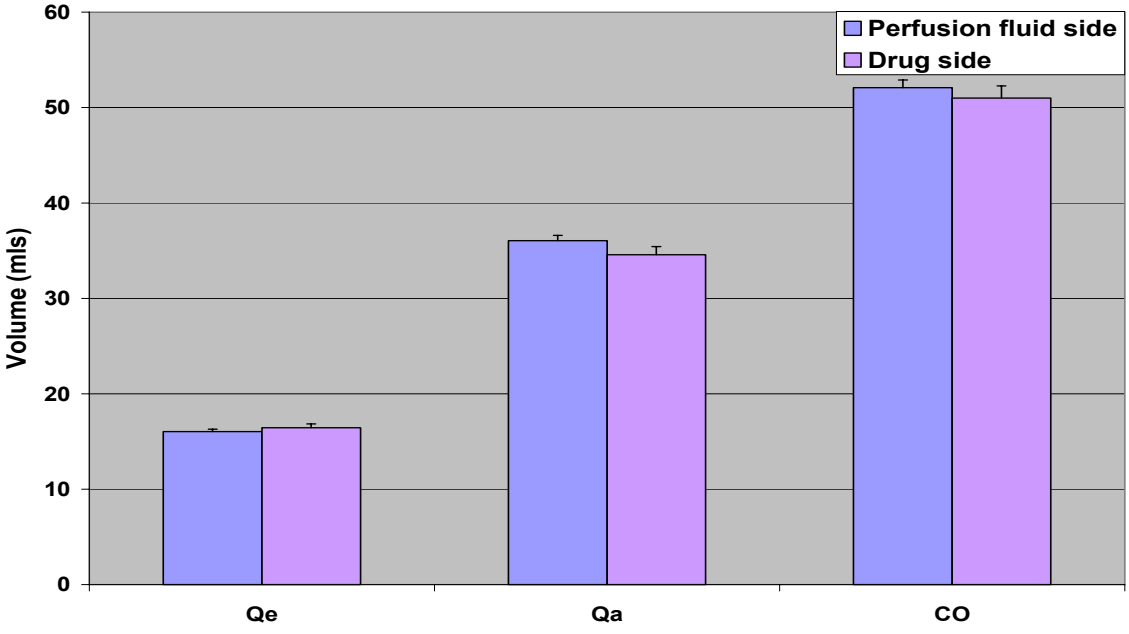

Figure 4.30: Coronary flow, aortic output and cardiac output readings for perfusion fluid and drug side of the double-sided working heart system.

4.2.1.3. Heart rate

Average heart rate for the 9 hearts was 234.75 beats/min and 236.5 beats/min for the perfusion fluid and drug side respectively (fig. 4.31). These means however did not differ significantly (p=0.6408), but fell within the recommended range for healthy hearts.

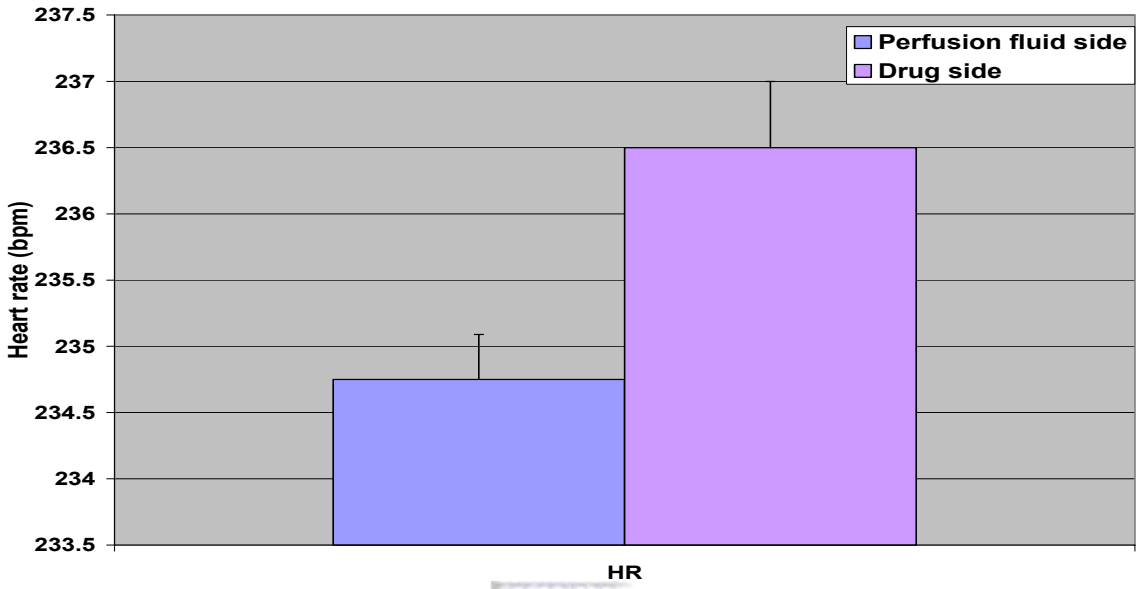

Figure 4.31: Heart rate readings for perfusion fluid and drug side of the double sided working heart system.

From the above results, both sides of the working heart system produced similar readings under similar circumstances (as indicated by the non-significant differences in the means of the different parameters), and so the system could be used to evaluate the test substances.

4.2.2 EFFECTS OF STANDARD DRUGS AND TEST SUBSTANCE

A sample size of 6 rats was used in evaluating the effect of each dose of standard drug and test substance administered. Readings were taking at the time of switching to working heart (drug/test substance) and also at the end of the 5-minute (drug/test substance) perfusion period. Results are displayed as a percentage change in readings taken just before switching to working heart (drug/test substance) perfusion and at the end of working heart (drug/test substance) perfusion.

4.2.2.4 Effect on force of contraction

Adrenaline

Adrenaline caused a dose dependent increase in the systolic pressure (from 4.5% ± 0.05 for a 0.001µg/ml to 11% ± 0.08 for a 1.0µg/ml solution). Between the doses 0.001µg/ml and 0.1µg/ml, the drug produced a decrease in diastolic pressure, with the greatest decrease of -7.9% ± 0.02 at the lowest dose (0.001µg/ml) and the lowest decrease of -0.7% ± 0.01 occurring at the dose of 0.1µg/ml. At the highest dose of 1.0µg/ml, there was an increase in diastolic pressure of 13.5% ± 0.04. Developed pressure decreases with an increase in dose from 29% ± 0.13 for 0.001µg/ml to 17% ± 0.08 for 1.0µg/ml (fig 4.32).

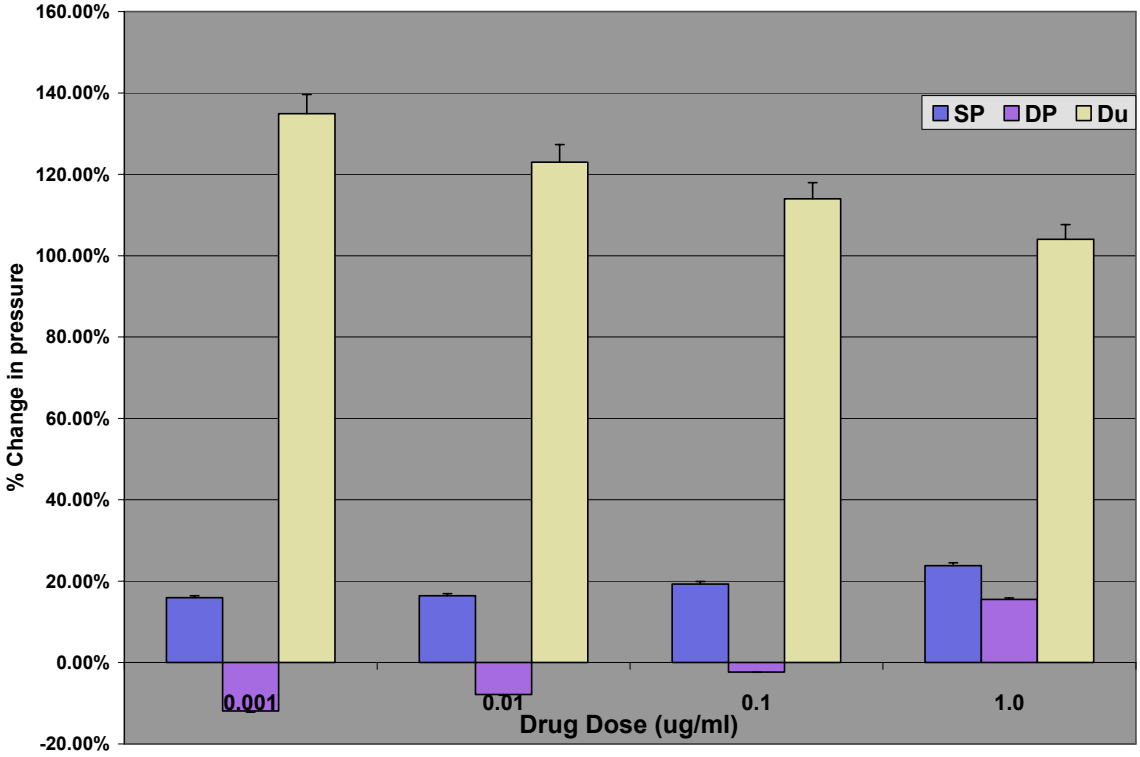

Figure 4.32: Effects of Adrenaline on systolic, diastolic and developed pressures.

The above results are indicative of the effects of adrenaline on the rate and force of contraction of the heart. Adrenaline acts on the β_1 receptors in the heart leading to an increase in the rate and force of contraction of the heart. Adrenaline also causes vasoconstriction in peripheral vascular beds, but in the case of the isolated perfused heart preparation as this, the increase in systolic pressure is a direct consequence of an increased chronotropic and inotropic effect.

Atenolol

Figure 4.33 below shows the effect of atenolol (0.01mg/ml – 0.1mg/ml) on systolic, diastolic and developed pressure. Atenolol produced a dose dependent decrease in systolic, diastolic and developed pressure. The smallest decrease in systolic pressure (-8.33% ± 2.34) occurred at the lowest dose (0.01mg/ml), while the highest decrease (-30.95% ± 3.5) occurred at the 0.07mg/ml dose. Further increases in the dose of atenolol did not produce any further increase in the decrease in systolic pressure beyond the 0.07mg.ml dose. The same changes occurred in diastolic pressure, with the smallest decrease (-5.09% ± 1.87) occurring at the lowest dose (0.01mg/ml) administered and the largest decrease (-22.03% ± 2.78) occurring at the 0.07mg/ml dose. As with systolic and diastolic pressure, developed pressure decreased by (-16.00% ± 3.45) at the lowest dose (0.01mg/ml), and maximum decrease (-53.00 ± 3.56.) occurred at 0.07mg/ml atenolol.

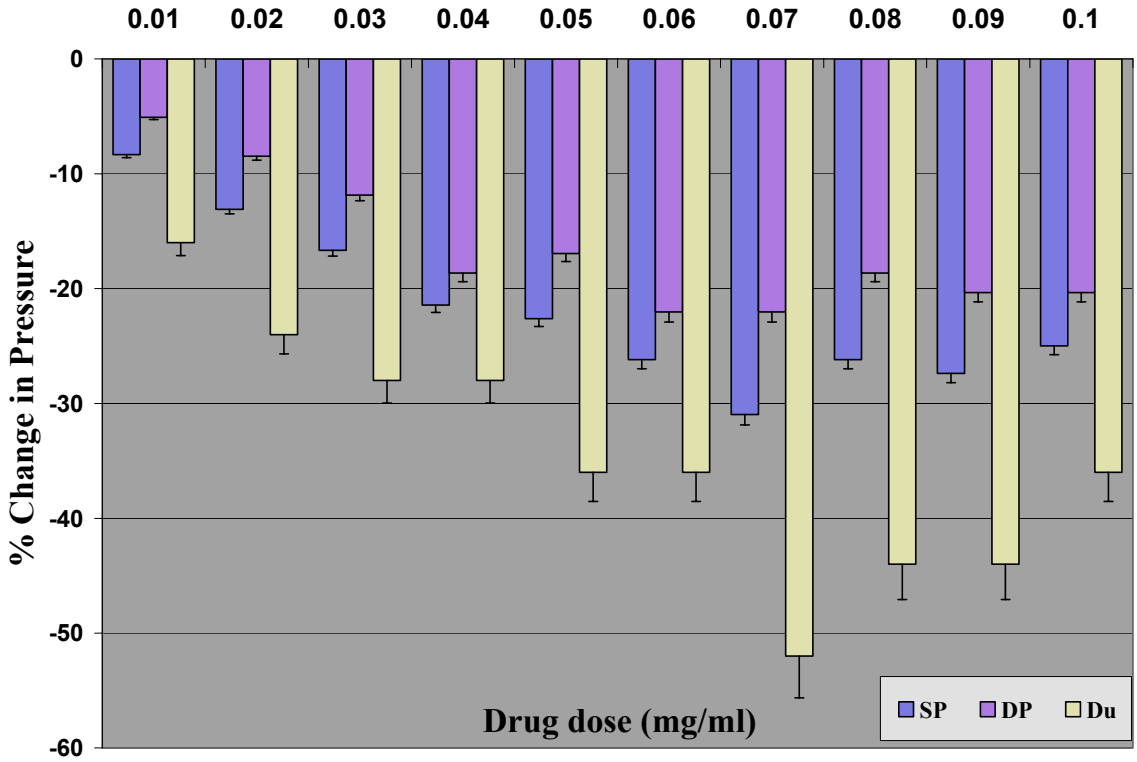

Figure 4.33: Effects of Atenolol on systolic, diastolic and developed pressures.

Atenolol acts selectively on the β_1 receptors in the heart. Its inhibitory actions leads to a decrease in heart rate, force of contraction and cardiac output, which in turn leads to a fall in blood pressure. In isolated perfused heart experiments where there are no blood vessels present, the reduction in systolic, diastolic and developed pressure as seen in figure 4.33 above is due to the reduction in heart rate, force of contraction and cardiac output by

atenolol. The 0.07mg dose of atenolol was used in combination with fraction C because it produced the peak response in parameters

Fraction C

Figure 4.34 shows the effect of a single dose of fraction C (0.01mg/ml) on the systolic, diastolic and developed pressures of the isolated heart. Systolic pressure increased by 12.89% ± 1.07, diastolic pressure decreased by 18.47% ± 1.1 and developed pressure increased by 88.34% ± 4.4. These changes were statistically significant (p= 0.0021, 0.0022 and 0.0021 for SP, DP and Du respectively).

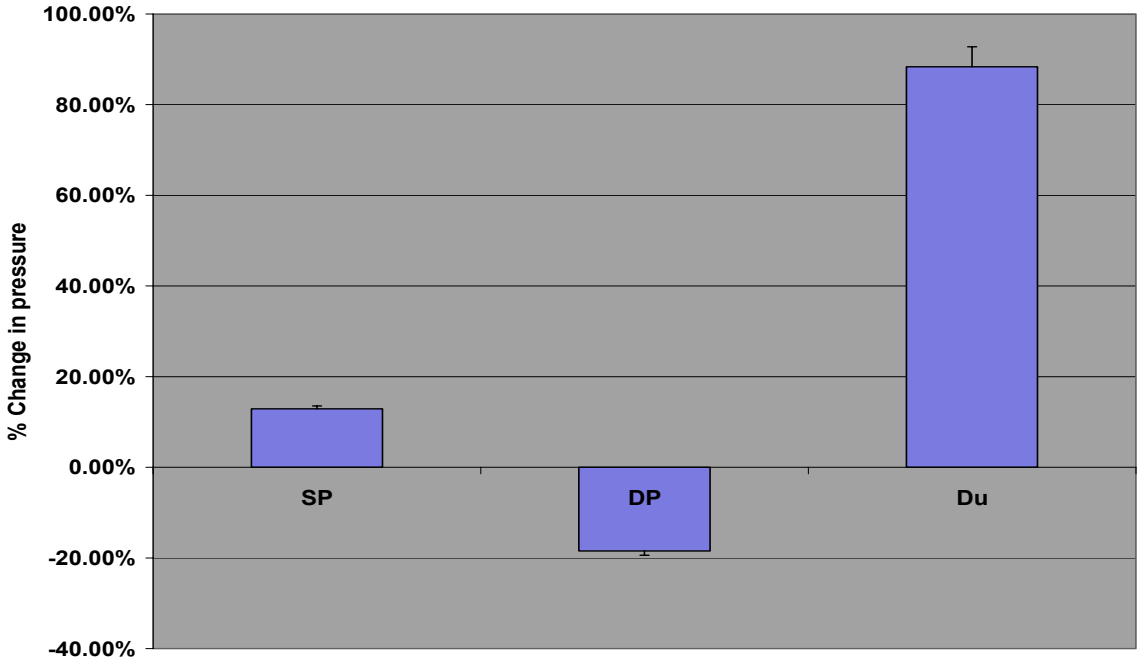

Figure 4.34: Effects of fraction C on systolic, diastolic and developed pressure.

The 0.01mg/ml solution of fraction C increased systolic and developed pressures while decreasing diastolic pressure. This effect on systolic, diastolic and developed pressure was similar to that observed upon adrenaline administration (see fig. 4.32), while the effect on systolic pressure and developed pressure are opposite that observed with atenolol administration (see fig. 4.33). The increase in systolic pressure could be as a result of an increase in the rate and force of contraction of the heart, while the decrease in diastolic pressure could be attributed to a decrease in the cardiac cycle due to an increase in the generation of action potentials and a decrease in conduction time through the AV node and Purkinje fibres. Developed pressure, which indicates the state of contractility of the cardiac muscles, was increased, indicating an increase in the rate and force of

contractility of the myocardium. The above results suggest that the effects of fraction C could be via stimulation of β_1 receptors in the heart.

Fraction C and atenolol

As shown in figure 4.35, the 0.01mg/ml solution of fraction C increased the systolic pressure by 15.87% ± 1.07, decreased diastolic pressure by -22.88% ± 1.19 and increased developed pressure by 107.33% ± 10.4. However, when administered with a 0.07mg/ml solution of atenolol, there was a significant reduction in the increase in systolic pressure (1.79% ± *0.76*) (*p*=0.0113). There was also a reduction in the increase in developed pressure (58.00% ± *3.45)*, though this was not statistically significant (*p*=0.1177). A slight reduction in the decrease in diastolic pressure was also recorded (-22.04% ± *2.19)*, though this also was not statistically significant (*p*=0.8983).

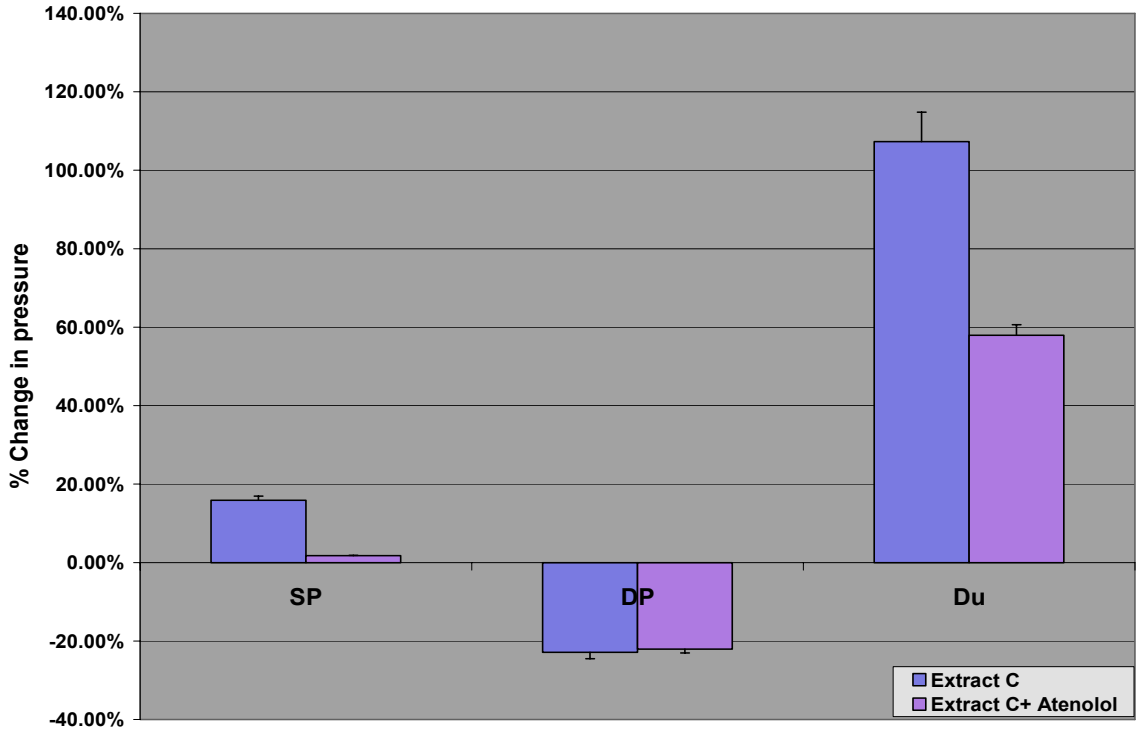

Figure 4.35: Effects of fraction C and a combination of fraction C and atenolol on systolic, diastolic and developed pressures.

Atenolol decreases the rate and force of contraction of the heart, leading to a decrease in systolic and diastolic pressures. As seen in figure 4.35 above, atenolol reduced the effect of fraction C as evident in the reduction in the increase in systolic pressure and developed pressure, as well as the reduction in the decrease in diastolic pressure. This suggests that atenolol inhibits the actions of fraction C, possibly on the β_1 receptors in the heart.

4.2.2.5 Effect on Coronary flow (Qe), Aortic output (Qa), and Cardiac output (CO).

Adrenaline

As seen in figure 4.36 adrenaline produced a dose dependent increase in coronary flow (21% ± 0.60, 26% ± 0.52, 30% ± 0.46 and 43% ± 0.54 for doses 0.001µg/ml, 0.01µg/ml, 0.1µg/ml and 1.0µg/ml respectively). Aortic output and cardiac output also increased in a dose dependent manner (26% ± 5.34 to 64% ± 6.17 for 0.001µg/ml to 1.0µg/ml, aortic output and 28% ± 1.03 to 66% ± 3.81 for cardiac output).

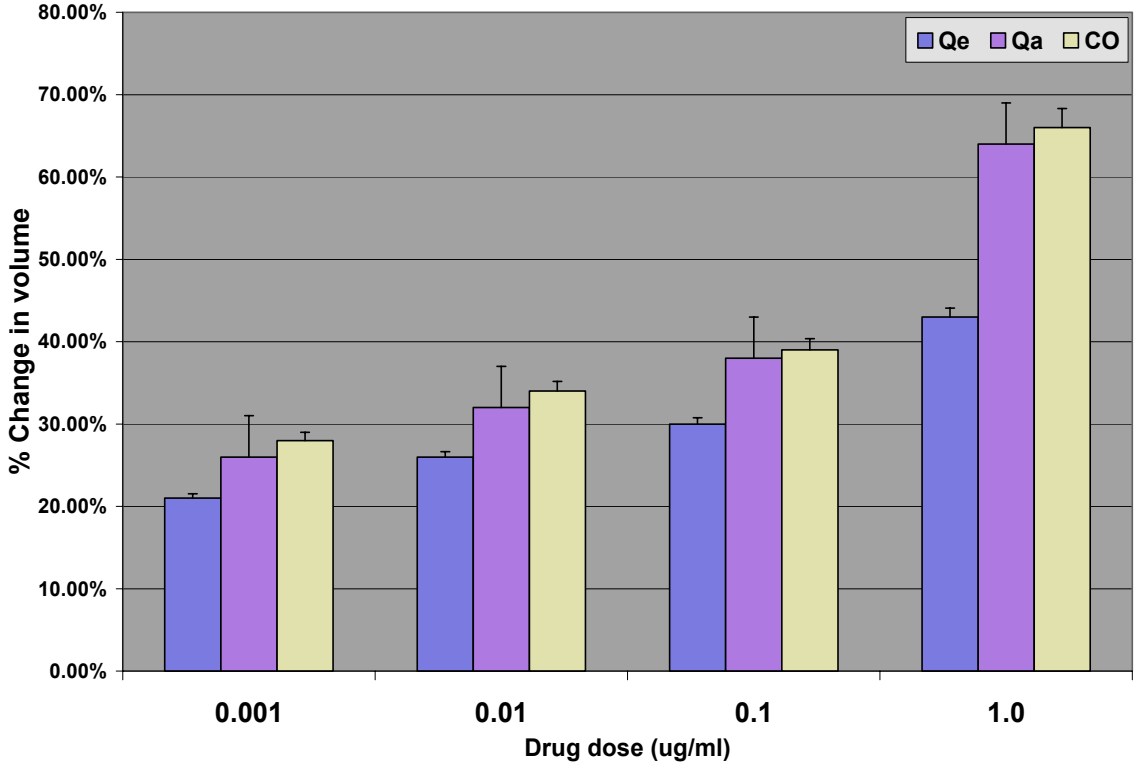

Figure 4.36: Effect of adrenaline on coronary flow, aortic output and cardiac output.

Coronary blood flow is enhanced by adrenaline, possibly by vasodilatation of the coronary vessels. In addition to this, adrenaline also reduces the refractory period between contractions, accelerates relaxation, and reduces the time taken for changes in intraventricular pressure (Katzung, B.G. 1998). This leads to positive chronotropic and inotropic effects and an increase in aortic output and cardiac output.

Atenolol

Figure 4.37 below shows the effect of atenolol (0.01mg/ml – 0.1mg/ml) on coronary flow, aortic output and cardiac output. Atenolol produced a dose dependent decrease in all the above parameters. The smallest decrease in coronary flow (-10.00% ± 2.45)

occurred at the lowest dose (0.01mg/ml), while the highest decrease (-80.65 ± 4.56) occurred at the 0.07mg/ml dose. Beyond the 0.07mg/ml dose, no further decreases in coronary flow occurred with further increases in the dose of atenolol administered. Similar dose dependent changes occurred with aortic output, with the smallest decrease (-15.39 ± 4.67) occurring at the lowest dose (0.01mg/ml) administered and the largest decrease (-100.39 ± 11.79) occurring at the 0.07mg/ml dose. The greatest decrease (-91.30 ± 8.90) in cardiac output occurred with the 0.07mg dose of atenolol also, while the smallest decrease (-13.04 ± 7.65) occurred at the lowest dose (0.01mg/ml).

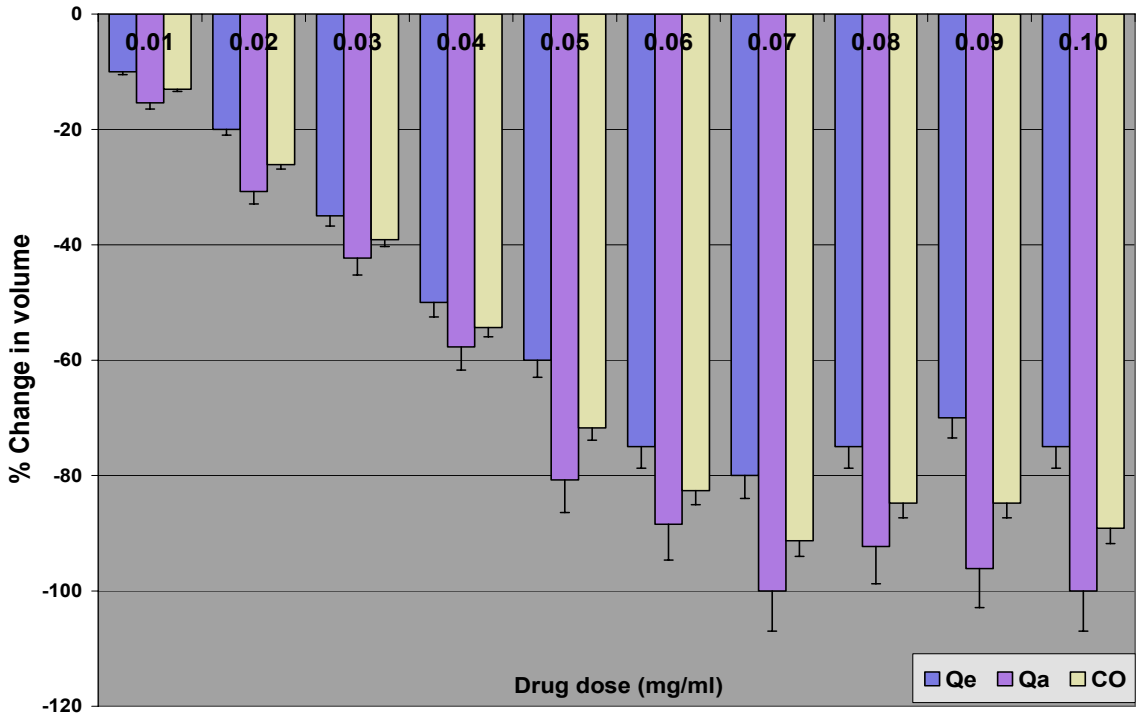

Figure 4.37: Effects of Atenolol on coronary flow, aortic output and cardiac output.

Atenolol selectively inhibits the β_1 receptors in the heart leading to a decrease in heart rate and force of contraction. This in turn leads to a decrease in aortic and cardiac output. The decrease in coronary flow probably is as a result of the decrease in cardiac output since the coronary vessels are fed via the aorta.

Fraction C

Figure 4.38 shows the effect of a 0.01mg/ml solution of fraction C on coronary flow, aortic output and cardiac output. Fraction C produced an increase in coronary flow (25.67% ± 0.9), aortic output (38.43% ± 1.3) and cardiac output (29.70% ± 1.0) similar to

that produced by adrenaline. All the changes in the parameters were statistically significant (p=0.011315, 0.0001, and 0.001134 for Qe, Qa and CO respectively).

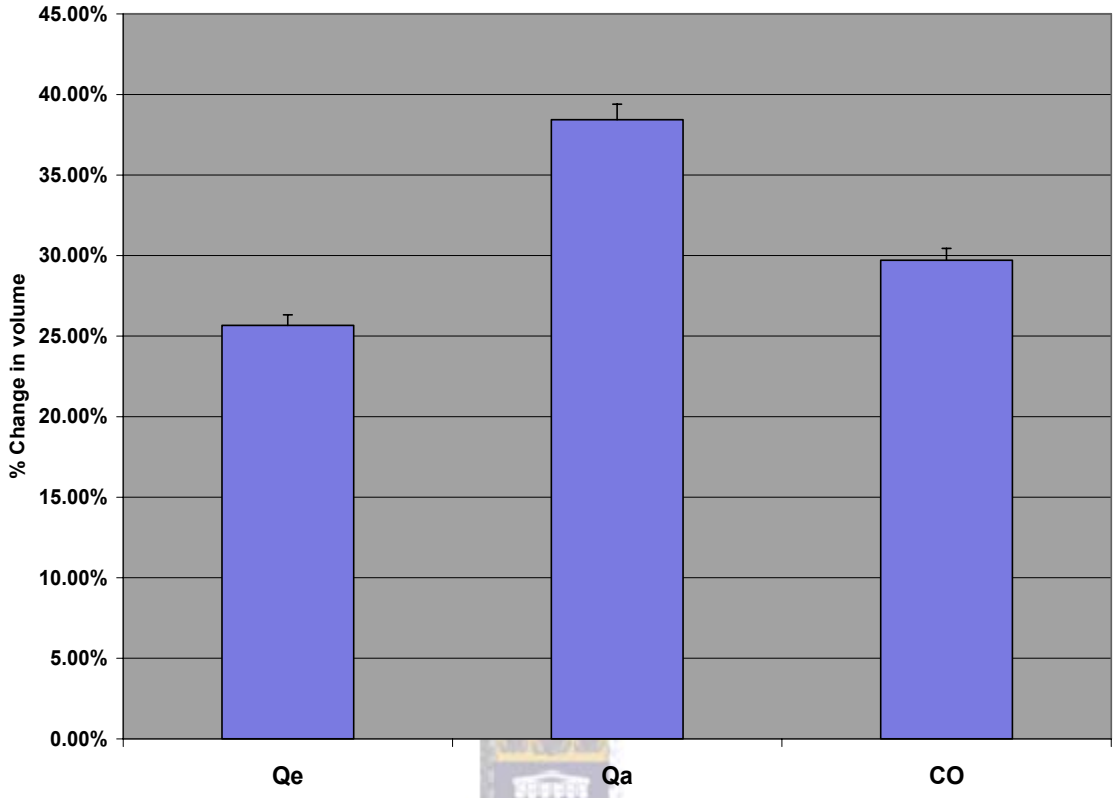

Figure 4.38: Effect of fraction C on coronary flow, aortic output and cardiac output.

The 0.01mg/ml solution of fraction C had an effect similar to that of adrenaline on coronary flow, aortic output and cardiac output. These effects could possibly be due to an adrenaline-like effect on the rate and force of contraction of the heart.

Fraction C and atenolol

As shown in figure 4.39 below, fraction C (0.01mg/ml) increased coronary flow by 29.17% ± 1.3, aortic output by 39.1% ± 2.6 and cardiac output by 34.78% ± 1.5. Co – administration with atenolol (0.07mg/ml) led to significant changes in these parameters. Coronary flow decreased by -4.17% ± 0.09 from an increase of 29.17% ± 1.3 (p=0.0001), aortic output decreased by -60.9% ± 3.2 from an increase of 39.1% ± 2.6 (p<0.0001) and cardiac output decreased by -36.23% ± 1.4 from an increase of 34.78% ± 1.5 (p<0.001) when fraction C was co-administered with atenolol.

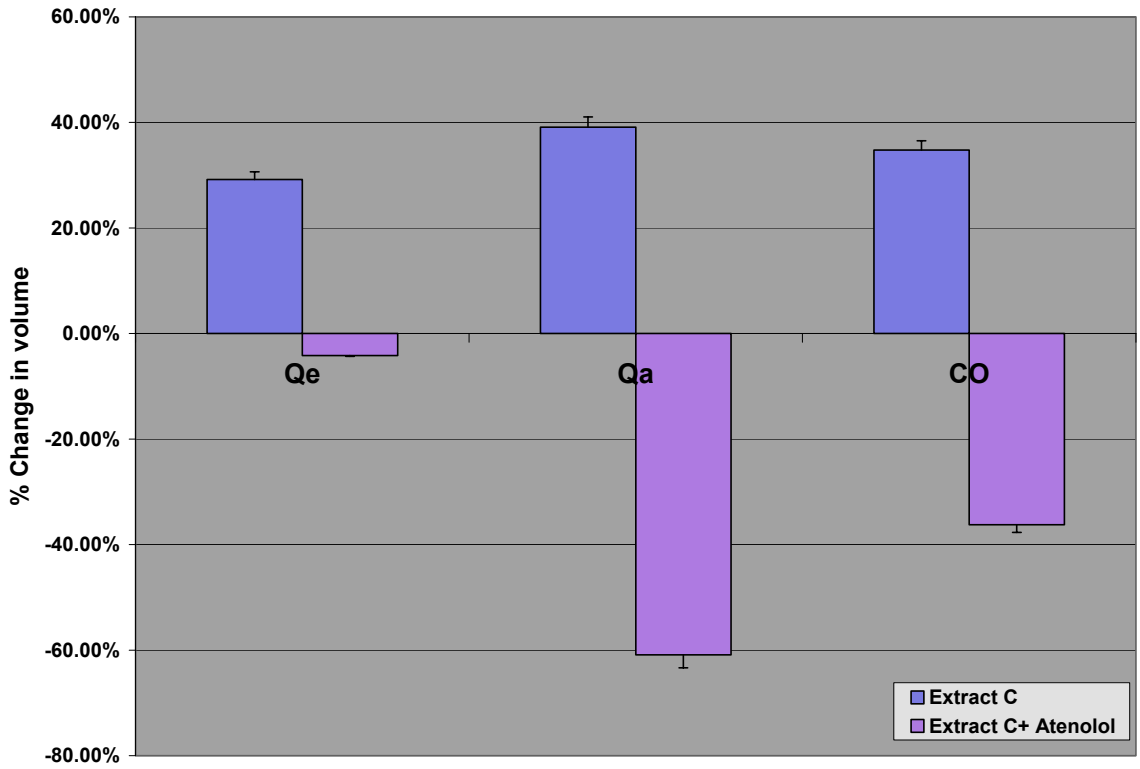

Figure 4.39: Effects of fraction C and the combination of fraction C and atenolol on coronary flow, aortic output and cardiac output.

Upon co-administration with atenolol, the effects of fraction C on the coronary flow, aortic output and cardiac output were reversed. This indicates that atenolol inhibited the actions of fraction C on the heart by blocking the β_1 receptors. This suggests that the effects of fraction C are mediated by the stimulation of the β_1 receptors in the heart.

4.2.2.6 Effect on Heart rate (HR)

Adrenaline

Adrenaline had a dose dependent effect on the heart rate (fig. 4.40). The lowest dose (0.001µg/ml) increased the heart rate by 8.7% ± 1.3, 0.01µg/ml increased the heart rate by 9.45% ± 1.2, 0.1µg/ml increased it further by 13.65% ± 1.6 and the highest dose 1.0µg/ml increased the heart rate by 19.7% ± 1.8.

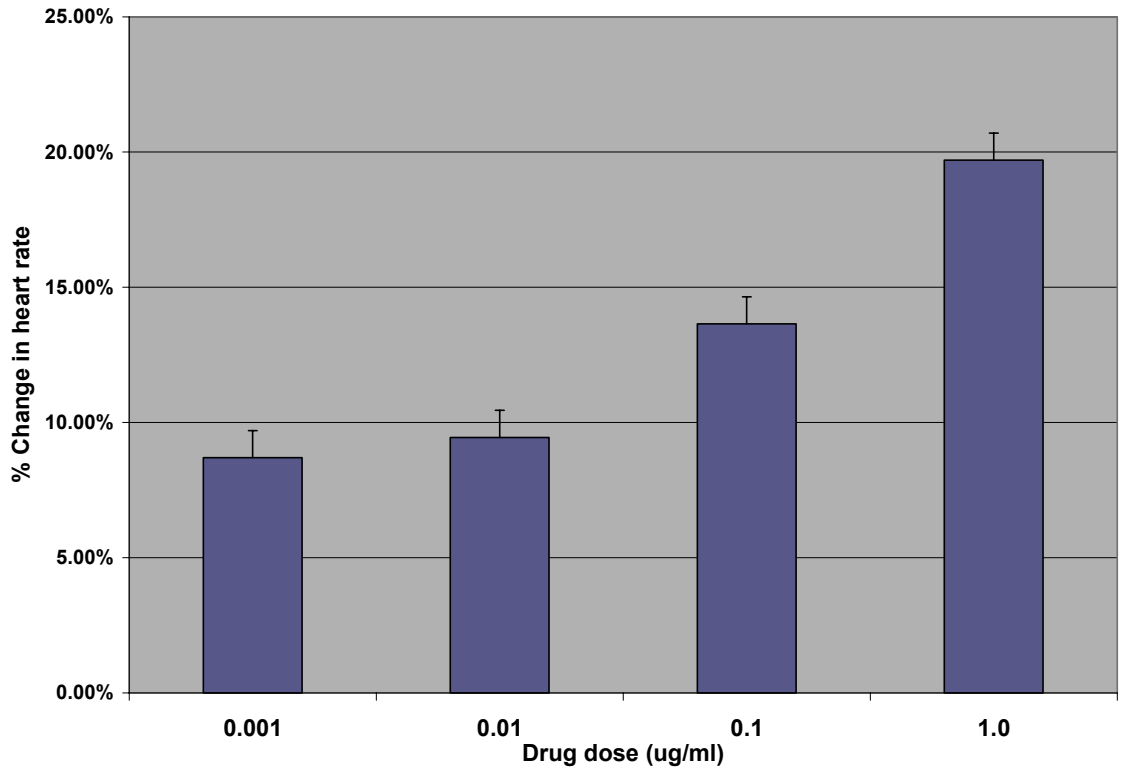

Figure 4.40: Effect of adrenaline on heart rate.

The effect of adrenaline on the heart rate as seen in figure 4.40 is due to the stimulation of β_1 and α_1 receptors in the heart, though adrenaline also acts on α_2 adrenoreceptors. Via its effect on β_1 receptors in the heart, adrenaline increases the rate contraction of the heart as seen in figure 4.40 above.

Atenolol

Figure 4.41 below shows the effect of atenolol (0.01mg/ml – 0.1mg/ml) on heart rate. Atenolol produced a dose dependent decrease in heart rate; with the smallest decrease (-18.91% ± 3.45) occurring at the lowest dose (0.01mg/ml), and the largest decrease (-53.36 ± 5.67) occurring at the 0.08mg/ml dose. Further increases in the dose of atenolol to 0.10mg/ml did not produce any further decrease in heart rate beyond that produced by the 0.09mg.ml dose.

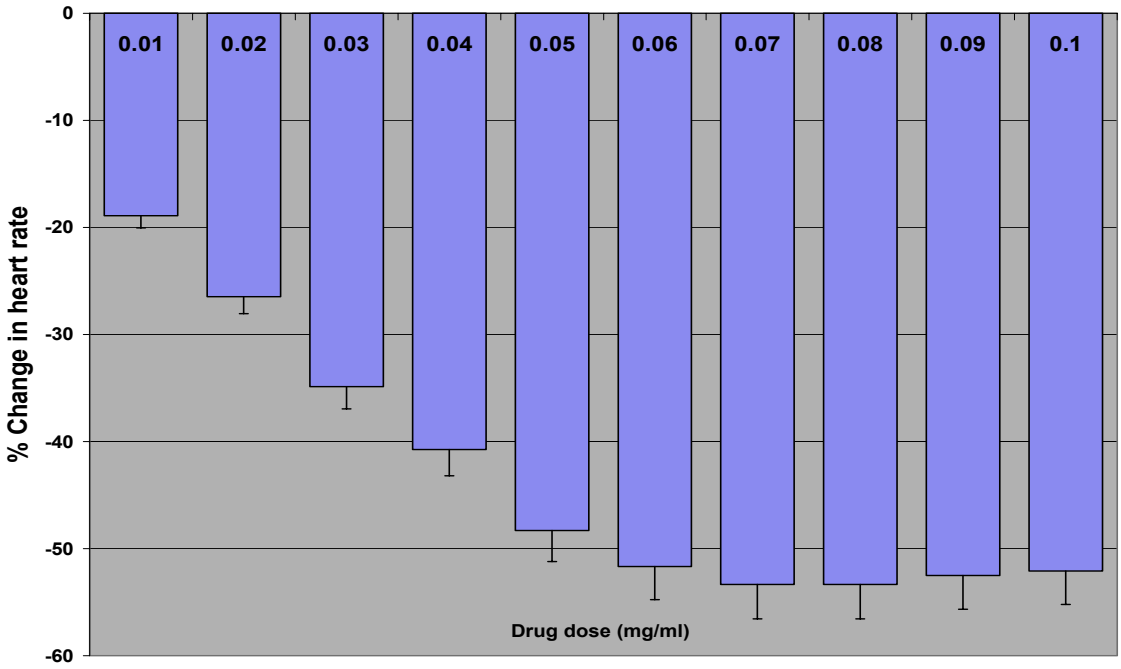

Figure 4.41: Effect of Atenolol on heart rate.

Fraction C

Fraction C given as a 0.01mg/ml solution produced an increase in the heart rate of 9.64% ± 0.4 which was statistically significant (p<0.001) (fig. 4.42).

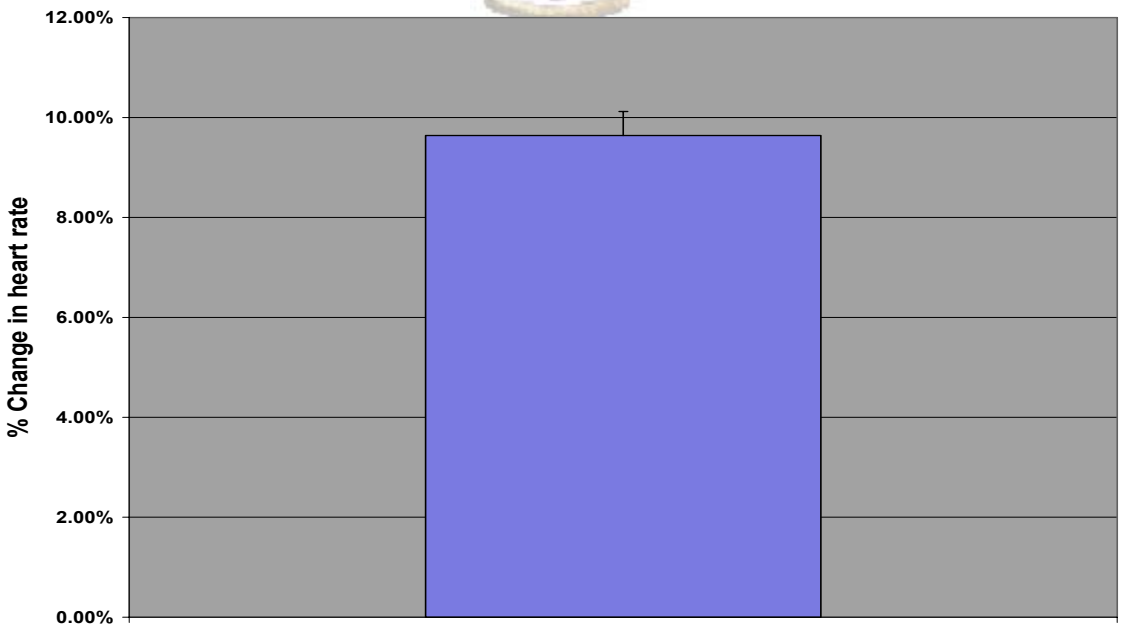

Figure 4.42: Effect of 0.01mg/ml fraction C on heart rate.

The 0.01mg/ml solution of fraction C had an effect on heart rate similar to the effect of adrenaline (see fig. 4.40), and opposite to the effect of atenolol (see fig 4.41). From this,

its is suggested that the effect of fraction C on the heart rate was due to a β_1 stimulant effect on the heart.

Fraction C and atenolol

When administered alone, fraction C increased the heart rate by 8.75 ± 0.45, however when co-administered with atenolol (0.07mg/ml), the effect was a 13.87% ± 1.04 decrease in heart rate (fig. 4.43). This difference in the effect on heart rate was statistically significant (p<0.0001).

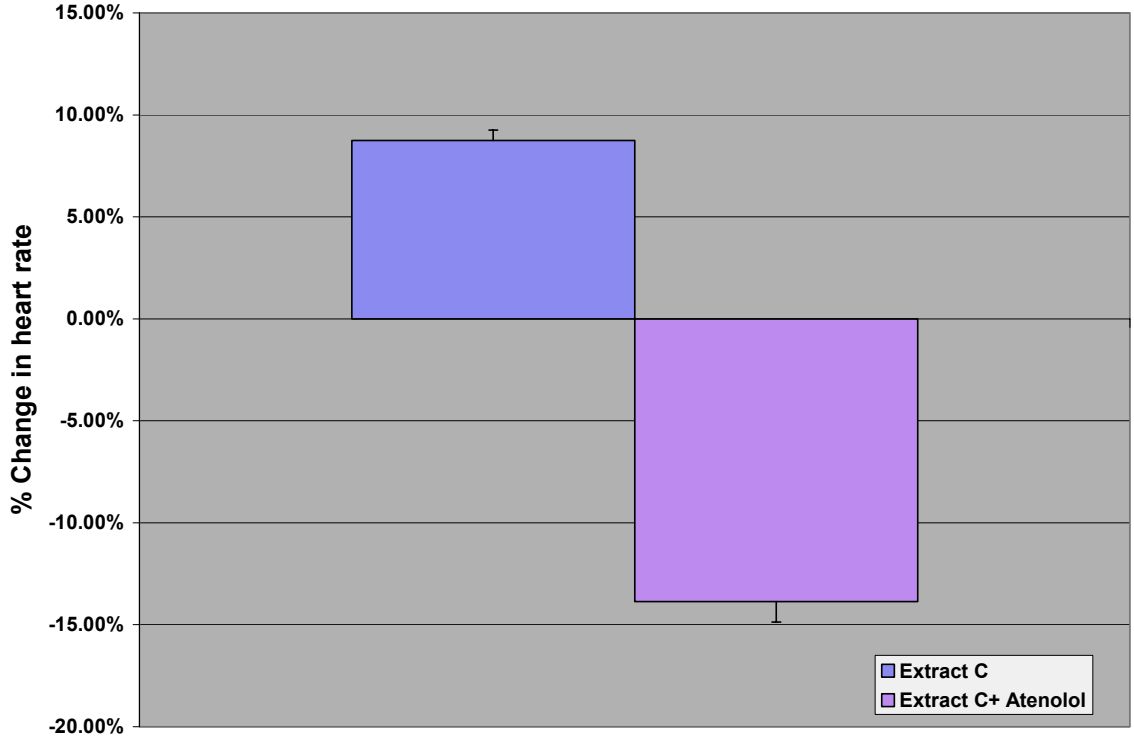

Figure 4.43: Effect of 0.01mg/ml fraction C, and the combination of 0.01mg/ml fraction C and 0.07mg/ml atenolol on heart rate.

Co-administration of atenolol completely reversed the positive chronotropic effects exhibited by fraction C. Heart rate was reduced, suggesting that both drugs acted via the same receptors in the heart.

4.3. DISCUSSION

Different drugs produce different effects on the different anatomical sites of the cardiovascular system. The action of a drug could be deciphered by its effect on measurable cardiovascular parameters like systolic pressure, diastolic pressure, mean arterial pressure, developed pressure, heart rate, coronary flow, aortic output and cardiac output.

4.3.1. *IN VIVO*

Adrenaline is an adrenergic agonist with effects on α and β receptors. At low doses, it produces a decrease in total peripheral resistance when administered I.V from a combination of α mediated vasoconstriction and β mediated vasodilatation, but at higher doses, the drug produces an increase in total peripheral resistance via α mediated vasoconstriction (Goldberg, L.I. *et al* 1960). In this study using anaesthetized normotensive rats, adrenaline produced an increase in systolic pressure, diastolic pressure, and mean arterial pressure at all doses. The drug also produced an increase in heart rate, a part of its positive chronotropic and inotropic effect via action on α and β receptors in the heart. Atenolol an adrenergic antagonist selective for the b_1 receptors in the heart would antagonise the positive chronotropic and inotropic effects of agonists like adrenaline. Its effects would also lead a decrease in systolic, diastolic and mean arterial pressures due directly to its inhibitory effects on heart rate and force of contraction. Prazocin is a selective α_1 receptor antagonist, and its inhibition of α_1 receptors in the vasculature would lead to vasodilatation and a decrease in systolic pressure. In intact animals, prazocin also produces reflex tachycardia indirectly by the activation of the barorecptor reflex. As such the decrease in systolic pressure is often accompanied by an increase in heart rate (Katzung, B (ed) 1998; Antonaccio, M (ed) 1977; Goldberg, L.l 1960). Adrenaline was used in this study to compare its effects with those of the test substances. Atenolol was employed for its selective β_1 antagonist effect, while prazocin was also used for its selective α_1 antagonist effect to, to locate possible mechanisms of action of the test substances.

The crude aqueous extract of *Leonotis leonurus* increased systolic pressure for all doses administered. This effect was similar to the effect of adrenaline on systolic pressure, but the opposite of the effect observed by Ojewole using a dose range of 25mg/kg – 80mg/kg in anaesthetized male wistar rats (Ojewole, J.A.O. 2003). Its effect on diastolic pressure

however was slightly different. Low doses (0.5 and 1.0mg) of the extract caused a decrease in diastolic pressure, while higher doses produced an increase, similar to that of adrenaline. Mean arterial pressure increased with an increase in dose of the extract. At low doses (0.5 and 1.0mg), the extract had a positive chronotropic effect on heart rate, while at higher doses, this effect was reversed to a negative chronotropic effect that increased with increase in dose.

Blood pressure is a function of cardiac output and peripheral resistance.

$$\mathbf{BP} \propto \mathbf{CO} \cdot \mathbf{PR} \qquad (4.1)$$

(Where BP = Blood pressure, CO = Cardiac output and PR = Peripheral resistance)

Cardiac output is directly related to heart rate, with an increase in heart rate leading to an increase in cardiac output.

$$\mathbf{CO} \propto \mathbf{HR} \cdot \mathbf{SV} \qquad (4.2)$$

(Where CO = Cardiac output, HR = Heart rate and SV = Stroke volume)

As can be inferred from equations 4.1and 4.2 above, an increase in heart rate would lead to an increase in cardiac output, which would lead to an increase in blood pressure if the peripheral resistance held constant or increases. Adrenaline produces an increase in blood pressure by increasing the heart rate (via its β_1 effect) and increasing peripheral resistance (via its α_1 vasoconstrictive effect). At low doses (0.5 and 1.0mg), the crude extract increased blood pressure (SP, DP and MAP), and this was thought to be due to its positive chronotropic effect on the heart (increase in heart rate). At higher doses however, though there was a decrease in heart rate, and an increase in blood pressure occurred. This was thought to be due to an increase in peripheral resistance by the crude extract.

Pre treating the animals with atenolol would lead to β_1 blockade and antagonism of any β_1 mediated effects by the crude extract. At low doses (0.5 and 1.0mg), the positive chronotropic effect of the crude extract was completely blocked and the net effect was a negative chronotropic effect. There was also a decrease in blood pressure at this dose, suggesting that the extract only acted on the heart at this dose. At the higher doses, the negative chronotropic effect of the extract was enhanced by pre-treatment with atenolol. Blood pressure was increased, though not to the same extent as that produced by the extract alone. This suggests that the extract increased blood pressure by some other means apart from increasing heart rate. A possible mechanism is vasoconstriction via the α_1 receptors in the blood vessels.

Prazocin a selective α_1 antagonist produces a drop in blood pressure by blocking α_1 receptors responsible for maintaining vascular muscle tone. With the pre administration of prazocin, the crude extract still produced an increase in systolic pressure, but this was less than the increase produced by the extract alone. A further decrease in diastolic pressure occurred at the lowest dose, while the slight increase produced by 1.0mg and 2.0mg of the crude extract was reversed. At higher doses, the increase in diastolic pressure was reduced by pre administration of prazocin. This suggests that the extract increased blood pressure at higher doses by vasoconstriction.

Fraction C increased systolic pressure, diastolic pressure, mean arterial pressure and heart rate in a manner similar to that of adrenaline at the doses tested. The increases in parameters were dose dependent suggesting that the fraction acted on the same receptors as adrenaline i.e. the β_1 receptors in the heart. Fraction C also increased heart rate in a dose dependent manner, also similar to that obtained with adrenaline suggesting that fraction C acted on β_1 receptors in the heart. Unfortunately, fraction C was not infused with the antagonists – atenolol and prazosin, and as such its mechanism cannot be actually ascertained. Comparing the effects of Fraction C to that of the crude aqueous extract, both extracts had similar effects on systolic pressure and mean arterial pressure. Fraction C produced a higher increase in systolic and mean arterial pressure, though its dose range was much lower than that of the crude aqueous extract. In fact, the highest dose of fraction C (0.05mg) was 10 times lower than the lowest dose of the crude aqueous extract (0.5mg), but yet produced an increase 6 times higher than that of the lowest dose of the crude aqueous extract. This was expected as we assume that the extraction process further purifies the sample, getting rid of inactive or even antagonistic substances present in the crude aqueous extract. Similar effects were noted with diastolic pressure; while the two lowest doses of the crude aqueous extract reduced diastolic pressure, all doses of fraction C increased diastolic pressure even though the doses where much smaller than those of the crude aqueous extract. With heart rate the effects were similar to that obtained with diastolic pressure. The two lowest doses of the crude aqueous extract increased heart rate, while other higher doses decreased heart rate, while all doses of fraction C increased heart rate. These results suggest that fraction C contains the same compound that produces the positive chronotropic effects noted in the low doses of the aqueous extract, and that in higher doses of the crude aqueous extract, other compounds present in the plant antagonize this effect. Another possibility is that the

active compound(s) present in fraction C is not soluble in water, and so would not be present in the aqueous extract.

4.3.2. *IN VITRO*

Adrenaline is a non-specific α and a β_1 adrenoreceptor agonist. At therapeutic doses, it causes an increase in systolic pressure, aortic output and cardiac output in the isolated heart via its positive chronotropic and positive inotropic effect on the heart. At low doses, it produces an increase in coronary flow via and β_2 receptor mediated vasodilatation, but at very high doses, coronary flow is inhibited by a combination of α_2 receptor mediated vasoconstriction, the physical compression of coronary vessels by the ventricles and by the shorter refractory period within which coronary flow can occur.

Atenolol also produces specific effects on the isolated heart. Between 0.01mg/ml and 0.07mg/ml, there was a dose dependent decrease in Systolic pressure, diastolic pressure and developed pressure. Coronary flow, aortic output and cardiac output also dropped in a dose dependent manner. Heart rate also decreased in a dose dependent manner and the drug can be said to have a negative chronotropic and negative inotropic effects on the heart. Atenolol slows down AV conduction and reduces automaticity at the SA and AV nodes, and also reduces contractile force in both atria and ventricles. These actions are via its inhibition of β_1 receptors in the heart.

The 0.01mg/ml solution of fraction C tested increased systolic pressure and developed pressure, effects due to its positive chronotropic effects on heart rate. This effect was similar to that observed by Mugabo and co-workers who noted a positive chronotropic and inotropic effect on isolated hearts perfused with a 1mg/ml solution of the crude aqueous extract (Mugabo *et al* 2002). Diastolic pressure was reduced due to the reduction in the time taken to complete the cardiac cycle, aortic output and cardiac output was increased due to the increase in heart rate. Coronary flow increased, either due to vasodilatation by fraction C or due to the increased heart rate. A vasodilatatory effect can however be ruled out considering the fact that in *in vivo* experiments, fraction C caused an increase in systolic pressure, not a decrease as would be expected with a vasodilatatory compound. The increase in developed pressure indicates an increase in the force of contraction – a positive inotropic effect on the heart (Seeley, R.R *et al* 2003). The net effect of this fraction was a positive chronotropic and positive inotropic effect on the isolated heart.

When co-administered with a 0.07mg/ml solution of atenolol, the effect of the fraction on heart rate was inhibited. The positive chronotropic effect was changed to a negative chronotropic effect. Systolic pressure and developed pressure decreased, while diastolic pressure increased. Coronary flow, aortic output and cardiac output also decreased. The inhibition of the effect of the fraction by atenolol suggests that its effects were via the β_1 receptors in the heart.

Compared to its effect in the anaesthetized normotensive rat, the 0.01mg/ml solution of fraction C produced a similar increase in systolic pressure and heart rate. For diastolic pressure, there was a decrease *in vitro*, while in the *in vivo* preparation there was an increase in diastolic pressure. The discrepancy in diastolic pressure is explained by the fact that in the *in vivo* system, diastolic pressure is measured in the peripheral circulation, while in the *in vitro* system, what is measured is end diastolic pressure in the heart itself. While peripheral systolic pressure gives an indication of peripheral resistance and cardiac output, end diastolic pressure gives a greater indication of the force of contraction of the heart (Berne, R.M *et al* 1986).

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1. CONCLUSION

Leonotis leonurus was chosen for this study because of its use in traditional medicine for the treatment of hypertension, and conflicting results from previous studies of its effects on the cardiovascular system. Previous studies by Mugabo and co-workers had reported that the plant had a positive chronotropic and inotropic effect on isolated rat hearts, while Njagi found no effect with a 1mg/ml solution on the isolated rat heart and in Ojewole's studies the extract (25mg/kg – 800mg/kg) decreased arterial blood pressure and heart rate (Mugabo *et al* 2002; Njagi *et al* 2001; Ojewole, J.O.A 2003). The aim of the study was to examine the effects of the plant in-vivo and in-vitro in a bid to reconcile the divergent views of previous studies, and to elucidate possible mechanisms of action of the plant using standard drugs. The dose dependent effect of the crude aqueous extract underlines the importance of the pharmacological screening of plants used in traditional medicine.

From the results obtained it can be concluded that:

a) *Leonotis leonurus* is a cardioactive plant. The crude aqueous extract of *Leonotis leonurus* and fraction C of the methanol extract of the leaves modified the cardiovascular parameters of the anaesthetized normotensive male Wistar rat and the isolated perfused rat heart.

b) The effect of the crude aqueous extract was dose dependent; a positive chronotropic effect at low doses (0.5 and 1.0mg) and a vasoconstrictive effect at higher doses (2.0 – 7.0mg) in anaesthetized normotensive male Wistar rats.

c) In anaesthetized normotensive male Wistar rat, fraction C of the methanol extracts had a positive chronotropic and positive inotropic effect.

d) In isolated working hearts, fraction C exhibited a positive chronotropic and positive inotropic effect.

5.2. RECOMMENDATIONS

Though this study adds to the scientific knowledge of the pharmacologic effects of Leonotis leonurus, it leaves a lot of questions yet unanswered on the effects of this interesting plant. More studies on the crude aqueous extract of the plant are needed to understand the dose dependent effects of the plant on heart rate and blood pressure. Further studies on the methanol fractions to determine their cardiovascular actions are required, and further work on the active fraction (fraction C) is needed to isolate and identify the active compound responsible for its effects. I also would recommend further studies on the mechanism of action of the crude aqueous extract with a view to determining the reasons for its dose dependent effect on heart rate.

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